CHARACTERIZATION OF BACTERIAL ISOLATES WITH DETECTION OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* AND EXTENDED SPECTRUM BETALACTAMASES PRODUCERS IN ADULT PNEUMONIA

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CERTIFICATE

This is to certify that this dissertation entitled **"CHARACTERIZATION OF BACTERIAL ISOLATES WITH DETECTION OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* & EXTENDED SPECTRUM BETALACTAMASES PRODUCERS IN ADULT PNEUMONIA"** is the bonafide original work done by **Dr. P. HEMA SUGANYA,** Post graduate in Microbiology, under my overall supervision and guidance in the Department of Microbiology, Govt. Kilpauk Medical College, Chennai, in partial fulfillment of the regulations of The Tamil Nadu Dr. M.G.R. Medical University for the award of **M.D Degree in Microbiology (Branch IV).**

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DECLARATION

I solemnly declare that this dissertation "**CHARACTERIZATION OF BACTERIAL ISOLATES WITH DETECTION OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* &EXTENDED SPECTRUM BETALACTAMASES PRODUCERS IN ADULT PNEUMONIA"** is the bonafide work done by me at the Department of Microbiology, Govt. Kilpauk Medical College and Hospital, Chennai, under the guidance and supervision of **Dr.RADHIKA KATRAGADDA, M.D.,** Professor & H.O.D of Microbiology, **Dr. K.V. LEELA, M.D., DGO.,** Professor, Department of Microbiology and **Dr. THYAGARAJAN RAVINDER, M.D.,** Professor, Department of Microbiology, Govt. Kilpauk Medical College, Chennai-600010. This dissertation is submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai in partial fulfillment of the University regulations for the award of Degree of M.D. Branch IV Microbiology examinations to be held in April 2017.

Place: Chennai. **Dr. P. HEMA SUGANYA**

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INTRODUCTION

Sir William Osler in year 1898 had described pneumonia in the elderly as "The friend of the aged, allowing them a merciful relief from those cold gradations of decay, that make the last state of all so distressing". Pneumonia is one of the most common infectious illness encountered in the clinical practice[1].

Pneumonia is an infection in lung parenchyma due to proliferation of pathogenic microorganisms at alveolar level and due to varied response of host towards these pathogens results in varied clinical symptoms [2].

In general population, infection that is frequent and accounting for larger number of working days lost is the respiratory tract infection[3]. Pneumonia being the major cause of morbidity worldwide mainly in developing countries with varied etiology pathogenesis, clinical presentation and epidemiology, early diagnosis of etiological agents and administration of specific antimicrobial treatment reduces complication and improves the prognosis. As per WHO estimation of adult pneumonia prevalence in South East Asia region, India accounts for about 4% [4]. Even though pneumonia accounts for more numbers of morbidity and mortality, it still has been underestimated, not properly diagnosed and treated[2].

Clinically pneumonia can be classified as Community acquired pneumonia (CAP) and Nosocomial pneumonia. Nosocomial pneumonia is further classified in to Hospital acquired pneumonia (HAP), Ventilator associated pneumonia(VAP) and Health care associated pneumonia (HCAP).

According to Infectious disease society of America (IDSA), Community acquired pneumonia (CAP) is defined as an acute onset of infection in the lung parenchyma associated with few symptoms, radiological evidence, auscultatory signs pertaining to pneumonia in patients who is not admitted in hospital or in any health care facility for greater than 14 days prior to the symptom onset[3]. Microorganisms causing Community acquired pneumonia includes *Streptococcus pneumoniae, Staphylococcus aureus, Haemophilus influenzae* and Gram negative bacilli such as *Klebsiella pneumoniae, Pseudomonas aeruginosa*[2]. Delay in diagnosis and associated risk factor of community acquired pneumonia may lead to complications like pleural effusion, lung abscess, bacteremia and empyema [4].

Hospital acquired pneumonia (HAP)- As per IDSA, pneumonia occurring after more than fourty eight hours of duration of stay in hospital without any previous symptoms of pneumonia [5]. Multidrug resistant pathogen (MDR) and polymicrobial infections associated with anaerobes is a complicating microbial etiology in HAP[2] .It increases the duration of stay in hospital of about seven to nine days per patient with also a economical burden to hospital[7].

Ventilator associated pneumonia (VAP) - Infection occurring in patients admitted in Intensive Care Unit (ICU) who is on mechanical ventilation for more than 48 hours of intubation [5,6,8,9]. Due to occurance of Multidrug resistant pathogen (MDR) such as MRSA, *Pseudomonas aeruginosa, Acinetobacter spp* and with inadequate antibiotic therapy results in poor patient prognosis which can be corrected by early diagnosis and administration of specific antibiotics at the earliest, so that morbidity and mortality rates can be lowered[9].

Health care associated pneumonia (HCAP)- Due to multiple risk factor in patients , they should be considered individually because of the emergence of multidrug resistant pathogen commonly MRSA. Recurrent pneumonia is a complication due to necrotizing infection of lungs[2].

In all categories of pneumonia, multidrug resistant pathogen includes Methicillin resistant *Staphylococcus aureus* (MRSA) in Gram positive isolates and Extended spectrum betalactamases (ESBL) producers in Gram negative bacilli has a major role in morbidity and mortality. Gram negative bacteria is the leading cause of pneumonia in the hospitalized patients due to longterm intake of antibiotics, surgery, trauma, malnourishment, tumor [10]. Ineffectiveness of antibiotics and increase in the severity of illness is due to quick spread of Extended spectrum betalactamases resistant pattern in to various pathogenic strain causing pneumonia[11].

Methicillin resistant *Staphylococcus aureus* (MRSA) expresses modification in pencillin binding protein (PBP2a) which leads to resistance of betalactam antibiotics. Change in resistance pattern of MRSA has lead to changes in antibiotic policy, health awareness with education and infection control measures which will reduce the disease burden of MRSA [12] .

Pneumonia remains a significant medical problem despite the advent of antibiotics, improved diagnostic and microbial techniques and sophisticated respiratory support system. Inorder to circumvent this problem due to resistance pattern of MDR isolates, gaining of knowledge about the local resistance pattern is

important to treat the infection with appropriate antibiotics and this can be achieved by conducting a local surveillance program[13].

This study aimed at studying the organisms distribution pattern in each categories of pneumonia by isolation, identification and characterization by standard microbiological technique with their antibiotic sensitivity pattern . The resistant organisms was screened for MRSA and ESBL followed by confirmation as per CLSI guidelines. Thus early diagnosis and effective treatment will reduce the local burden of the disease pattern, complications of pneumonia and knowing the antibiotic sensitivity pattern will help to formulate an antibiotic policy institutional patient care.

AIMS AND OBJECTIVE

1. To isolate, identify and characterize bacteria from adult patients with pneumonia.

2. To find out the antibiotic susceptibility pattern of the bacterial isolates.

3. To detect the Extended Spectrum Beta Lactamases (ESBLs), Metallobetalactamases, AmpC Beta Lactamases producers from Gram negative bacterial isolates.

4. To find the prevalence of Methicillin Resistant *Staphylococcus aureus* (MRSA) among *Staphylococcus aureus* isolates.

REVIEW OF LITERATURE

About 2500 years ago Hippocrates described pneumonia and Sir William Osler said that pneumonia as "Captain of the men of death". Pneumonia is an infection in lung parenchyma due to proliferation of pathogenic microorganism at alveolar level and due to varied immune response of host towards these pathogens, it presents as clinical symptoms [2].

EPIDEMIOLOGY:

Approximately more than six lakh individual admitted in hospital and 45 thousand death due to Pneumonia observed worldwide annually. Pneumonia is the 5th leading cause of death in the world[14]. Estimated prevalence of Pneumonia affecting adults in India is about 4%[4]. Due to the association with risk factor such as COPD, smoking, males are commonly affected and people between age group of 51-60 yrs are also affected due to chronic ailments.

In India, mortality rate of Community acquired pneumonia CAP lies between 3.3-11%[4]. Hospital acquired pneumonia (HAP) accounts for about 13-18% of all nosocomial infection and affects only 0.5- 2%[15] of hospitalized patients with mortality rate from 20-50%. (Hoffkenet al)[16,17]. In the study done by Vasuki et al[18] in Tamilnadu, the incidence of HAP is 10.3%. Patient on mechanical ventilation have rates of pneumonia 7 to 21 folds higher than patient not on ventilator support. Rate of infection is twice high in teaching institution when compared to small institution. Ventilator associated pneumonia (VAP), overall incidence in ICU ranges about 15-40%[19] and mortality rate is about 40-50% [20, 21, 22].

CAUSATIVE ORGANISMS OF PNEUMONIA[23]:

Bacteria - *Streptococcus pneumoniae, Haemophilus influenzae, Mycoplasma pneumoniae, Chlamydophila pneumoniae, pseudomonas aeruginosa, Enterobacteriaceae, Peptococcus, Prevotella, Actinomyces, Nocardia spp, Coxiella burnetii, Mycobacteria spp.*

Virus - *Respiratory syncytial virus, Influenza, Parainfluenza virus type1,2,3. Rhinovirus, Human Metapneumovirus, Adenovirus (type 4and7).*

Parasites – *Paragonimus westermani, Ascaris lumbricoides, Strongyloides stercoralis, Toxoplasma gondii.*

Fungi – *Histoplasma capsulatum, Coccidioides immitis, Mucor spp, Pneumocystis jirovecii, Rhizopus spp, Absidia spp.*

PATHOPHYSIOLOGY [2]:

The lung has been frequently exposed to the particulate things, gaseous mixture and numerous microorganism present in inspired air. In addition, seeping down of oral secretions from the upper respiratory tract occurs which leads to microaspiration. The lower respiratory tract are maintained sterile due to defense mechanisms of the respiratory tract (nasal hair, mucociliary clearance, gag reflex, cough mechanism).

The acute pulmonary infection is developed due to either a defect in host defenses, exposure to a particularly virulent microorganism .Infectious agents gain entry to the lower respiratory tract through aspiration of upper airway resident flora, inhalation of aerosolized material and less frequently, metastatic seeding of the lung

from blood. Alveolar macrophages ingest the pathogens and initiate an inflammatory response which triggers the clinical symptoms of pneumonia.

PATHOLOGY[2,24] :

Classic pneumonia evolves through a series of pathologic changes.

- Congestion /Edema- with the presence of a proteinaceous exudates, and bacteria in the alveoli.
- Red hepatization - presence of erythrocytes, neutrophils in the cellular intraalveolar exudate . During this phase, occasionally bacteria may be seen in the culture.
- Gray hepatization- no new extravastion of erythrocyte and those already existing are lysed and degraded. Neutrophil is the predominant cell, abundant fibrin deposition, and absence of bacteria which is sign of improving from infection.
- Resolution -The dominant cell type in the alveolar space is the macrophage, and bacteria, fibrin, debris of neutrophils has been cleared.

Because of the microaspiration mechanism, bronchopneumonia pattern is mostly seen in nosocomial pneumonias, whereas a lobar pattern in bacterial CAP.

RISK FACTORS [2, 25, 26]:

With advanced age group, smoking, alcohol consumption and the presence of coexisting illness like COPD, diabetes, chronic lung disease, renal failure, congestive cardiac disease and neurological illness, influence the outcome of

disease in CAP[25]. Addition to these prolonged intakes of antibiotics and increased hospital stay influences the outcome of disease in nosocomial pneumonia.

CLASSIFICATION OF PNEUMONIA:

Pneumonia may be classified according to its anatomical location in the lung[24]:

- Lobar pneumonia occurs in one part, or lobe, of the lung.
- Bronchopneumonia tends to be scattered throughout the lung.

Clinically pneumonia can be classified as per place of acquisition, Community acquired pneumonia (CAP) and Nosocomial pneumonia. Nosocomial pneumonia is further classified in to Hospital acquired pneumonia (HAP), Ventilator associated pneumonia (VAP) and Health care associated pneumonia (HCAP)[2].

1. COMMUNITY ACQUIRED PNEUMONIA:

CAP can be defined both on clinical and radiographic findings[27]. According to Infectious disease society of America(IDSA), Community acquired pneumonia(CAP) is defined as an acute onset of infection in the lung parenchyma associated with few symptoms, radiological evidence, auscultatory signs pertaining to pneumonia in patients who is not admitted in hospital or in any health care facility for greater than 14 days prior to the symptom onset[3].

CASE DEFINITION OF CAP[25, 28, 29]:

New or progressive infiltration in Chest Xray with atleast any two of the following symptoms: cough (>4 weeks), purulent sputum production, fever (temp.>37.8ºC) or total WBC count >10,000/mm^3.

ETIOLOGY AGENTS IN CAP[2]:

Outpatient setting- *Streptococcus pneumoniae, Haemophilus influenzae, Mycoplasma pneumoniae, Chlamydophila pneumoniae and* other respiratory viruses [*Respiratory syncytial virus, Influenza, Parainfluenza virus type1,2,3. Rhinovirus, human metapneumovirus, Adenovirus (type 4and7)].*

Inpatient setting-

NON –ICU-- *Streptococcus pneumoniae, Haemophilus influenzae, Chlamydophila pneumoniae ,Mycoplasma pneumoniae, Legionella pneumophila.*

ICU- *Streptococcus pneumoniae, Legionella pneumophila, Haemophilus influenza, Staphylococcus aureus*, Gram negative bacilli.

The causative bacterial agents of CAP in India varies with geographical distribution.for example, the leading causative agent in Shimla and Delhi is *Streptococcus pneumoniae* whereas in Ludhiana *Pseudomonas aeruginosa* predominates [25].

2. HOSPITAL ACQUIRED PNEUMONIA:

HAP is an inflammation of lung tissue by a pathogen neither present nor at incubation period during the time of hospital admission[27].As per IDSA, pneumonia occurring after more than fourty eight hours of duration of stay in hospital without any previous symptoms of pneumonia[6]. Due to HAP, duration of hospital stay is increased by an average of 7-9 days per patient with increase in financial burden[7].

CASE DEFINITION OF HAP[27, 30]:

New or progressive infiltration in Chest X-ray with atleast any two of the following symptoms: cough (>4 weeks), purulent sputum production, fever (temp.>37.8ºC) or total WBC count >10,000/mm^3 occuring in patients admitted in hospital for after more than fourty eight hours without any prior symptoms of pneumonia.

ETIOLOGY AGENTS IN HAP[31, 32, 33]:

Streptococcus pneumoniae, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Proteus spp, Serratia marcescens, Haemophilus influenza.

3. VENTILATOR ASSOCIATED PNEUMONIA[34]:

Ventilator associated pneumonia(VAP)-Infection occurring in patients admitted in Intensive Care Unit(ICU) who is on mechanical ventilation for more than 48 hours of intubation[8, 7]. VAP can be acquired in many routes including aspiration of oropharyngeal organism, hematogenous spread, MRSA and MDR pathogens from hands of health care workers, contaminated medical equipment[7] .

Classification of VAP[7,8] :

Based on duration of mechanical ventilation [19],

A. Early onset VAP- Occurs in first four days on ventilator with possible causative agents being Enterobacteriacea and *Staphyococcus aureus* ,carries better prognosis.

B. Late onset VAP- occurs in patient with five days or more on ventilator mainly due to nonfermenting GNB associated with MDR pathogens. mortality and morbidity rate is high.

Estimated risk of VAP with hospital stay is high in early as 3% /day for first five days of ventilation, 2% / day during 5-10 days of ventilation and 1%/day after 10 days of ventilation[7].

Case definition of VAP [13,35]:

Patient is on mechanical ventilation for more than 48 hours with suspected VAP ,

New and persistant chest infiltrate in chest radiograph with any of these 2 criteria:

- Fever (temp.>38ºC) or hypothermia (<36 ºC).
- WBC Count ≥10000 mm^3 or ≤ 4000mm^3.
- Purulent tracheal secretion.

ETIOLOGY AGENTS IN VAP [2]:

MDR Pathogens- MRSA, *Pseudomonas aeruginosa, Acinetobacter baumanii*, ESBL-positive strains *Klebsiella pneumoniae,* Antibiotic-resistant Enterobacteriaceae, *Legionella pneumophila, Enterobacter* spp, *Burkholderia cepacia.*

Non-MDR Pathogens - *Streptococcus pneumonia,* MSSA, *Klebsiella spp, Haemophilus influenza, Escherichia coli, Proteus* spp, *Serratia marcescens, Aspergillus* spp.

Clinical criteria of VAP[7, 8, 36, 37, 38]:

The Pugin's modified Clinical Pulmonary Infection Score (CPIS), which combines clinical, radiographic, physiological and microbiological data into a single numerical result, is used as a diagnostic tool for VAP. Modified CPIS > 6 is considered as diagnosis of pneumonia.

Microbiological Criteria of VAP[37]:

>25 PMN cells with few epithelial cells in Gram stained smear of ETA sample and Significant quantitative culture (colony count $\geq 10^5$ cfu/ml).

4.HEALTH CARE ASSOCIATED PNEUMONIA(HCAP)[39]:

Patient admitted in hospital for ≥2 days within 90 days of diagnosis of pneumonia, receiving I.V. antibiotics, residing in health care facility <30 days or received hemodialysis. Multidrug resistant pathogens are the major causative organism.

CLINICAL FEATURE OF PNEUMONIA[2]:

Symptoms - fever, cough (productive/non productive), shortness of breath, pleuritic type of chest pain.

Signs - tachycardia, tachypnoea, dull note on percussion, rales/ronchi/diminished breath sounds on auscultation.

Criteria for hospital admission:

To provide an efficient patient care, criteria has been laid down for patient in need of hospital care.

1.**Pneumonia Severity Index (PSI) scoring:**

The pneumonia severity index (PSI) was developed to categorize patients for hospital care [39].

Patient characteristic	**Points**
Demographic	
Age(years):	
Male: age	-
Female: age	-
Nursing home resident	+10
Co- morbidities	
Neoplastic disease	+30
Liver disease	+20
Congestive heart failure	+10
Cerebrovascular disease	+10
Renal disease	+10
Examination findings	
Altered mental status	+20
Respiratory rate 30/minute	+20
Systolic blood pressure <90 mmHg	+20
Temperature <35ºC or 40ºC	+15
Pulse rate125/minute	+10
Laboratory findings	
pH <7.35 (do ABG only if hypoxic or COPD)	+30
BUN >10.7 mmol/ L	+20
Sodium <130 mEq/L	+20
Glucose 13.9 mmol/L	+10
Hematocrit <0.30	+10
PaO2 <60mmHg or oxygen saturation <90%	+10
Pleural effusion	+30

Patients with a higher risk are defined as being in PSI risk class V (PSI-V) PSI score > 130.

2.CURB 65 Criteria: Confusion, **U**rea(>7mmol/L), **R**espiratory rate ≥30/mt, **B**lood pressure systolic ≤ 90mmHg or diastolic ≤ 60mmHg, **A**ge(≥65 years).

COMPLICATION OF PNEUMONIA[24]:

- Abscess formation particularly seen in type 3 *pneumococci, Klebsiella pneumoniae* , *Staphylococcus aureus*.
- Bacterial dissemination to brain, kidney ,spleen and joints.
- Empyema - infection spreading to pleural cavity and most common with infection of *pneumococcus*[40].

LABORATORY DIAGNOSIS[4]:

Total Leucocyte Count, Differential Count, Erythrocyte sedimentation rate , Sputum Microscopy for Gram Stain, Acid Fast Bacilli, Sputum culture and sensitivity, Chest X-ray PA view, Computerised Tomography Thorax (if necessary), Arterial blood gas analysis(VAP), Biomarkers-C-Reactive protein, Procalcitonin, sTREM-1(soluble triggering receptor expressed on myeloid cells)[5].

MICROBIOLOGICAL INVESTIGATION

COLLECTION OF SPECIMEN[41] :

1. SPUTUM[42, 43, 44]:

Sputum is a mixed collection of bronchial secretion and inflammatory exudates from affected lobe of lung parenchymal tissue that is coughed up in to the mouth and expectorated. Sputum should be differentiated from saliva, as sputum is purulent, opaque, viscus and yellow to green coloured whereas saliva is clear and watery in nature.

Instruction for sputum collection [41,45,46]:

1. Sputum is collected prior to the administration of antibiotics.
2. Sputum is collected in the early morning as soon as the patient awakes and asked to brush the teeth and then rinse the mouth with water before collection.
3. Sputum is collected in a disposable, sterile, wide mouthed, screw capped plastic container of about 100ml capacity.
4. Sputum can be collected by asking the patient to deeply cough out the sputum spontaneously or induced by administering saline nebulisation, postural drainage or by appropriate physiotherapy.
5. Sputum sample collected in the container is transported to laboratory for processing within 2 hours of collection.

Processing of sputum:

Homogenisation:

Purulent part of the sputum has the appropriate pathogen that is overlaid by clear to mucoid secretion. Homogenisation is done to make uniform mixture of the relavant pathogen to be present in the sample , so as each drop of the sample will contain some amount of pathogen that can be suitable for smear preparation and culture.

a. Equal volume of sputum sample and sodium dithiothreitol is mixed in a vortex mixture for about fifteen seconds and allow it to stand for about fifteen minutes in room temperature.

b. Equal volume of sputum sample and buffered pancreatin solution is mixed continuosly and gently in a machine that tilt to and fro and incubated at 37ºC for thirty minutes.

2) ENDOTRACHEAL ASPIRATE (ETA)[6,45,47]

Endo Tracheal aspirate (ETA) was collected from the patient who has been in mechanical ventilation for more than fourty eight hours with suspected VAP. One milliliter of ETA was collected in a sterile screw capped plastic container by using twenty two inch Ramson's 12 F suction catheter with a mucus extractor was introduced slowly in to the endotracheal tube for about a distance of twenty five to twenty six centimeter.

3) BRONCHOALVEOLAR LAVAGE (BAL)[48]:

30-50ml of sterile saline is injected in to the fibreoptic bronchoscope which is threaded to peripheral bronchiolar ramification and it is aspirated, collected in a sterile container.

4) BLOOD[45,48] :

Blood culture was performed in all cases of suspected pneumonia with fever (temp.>37.8ºC) prior to starting antibiotics. Sterile gloves were worn prior to the procedure and a patch of skin prepared approx. 5-cm in diameter over the proposed veni-puncture site. This area was cleansed thoroughly with 70% isopropyl alcohol, followed by povidone iodine, and followed again by 70% isopropyl alcohol in a concentric circles moving outward from the centre.

The skin was allowed to dry for at least 1 minute before the sample is collected 5 ml sample of blood was collected by venipuncture using sterile syringe

and transferred after removing the needle into the blood culture bottle containing 50 ml of Brain heart infusion broth, maintaining sterile aseptic precautions.

Direct Microscopy:

Smear is made from the homoginsed or purulent material of the sputum. Gram staining was done and examined under oil immersion field for the relative number of squamous epithelial cells and neutrophil.

BARLETT'S GRADING:

Number of neutrophil (lpf)	Grade
<10	0
10 – 25	+1
>25	+2

Number of epithelial cells	**Grade**
10 - 25	-1
>25	-2

Average number of neutrophils and epithelial cells for 20-30 LPFs was calculated and the total score arrived. A score of 0 or lessthan 1 is indicative of contamination and a score of 1 and above was considered an acceptable quality of sample.

MURRAY AND WASHINGTON GRADING[48]:

	Epithelial cells/lpf	Leukocytes /lpf
Group 1	25	10
Group 2	25	10 - 25
Group 3	25	25
Group 4	10 – 25	25
Group 5	<10	25

Only Group 5 specimen is accepted for culture.

An acceptable ETA samples in Gram stained smear shows less than ten squamous epithelial cells per low power or organisms under oil immersion field. Best ETA samples in Gram stained smear showed >25 polymorphonuclear leucocytes per low power field with minimal squamous epithelial cells[8,45]

3. CULTURE[41,45,48]:

Sputum samples were then plated into the following agar media: Nutrient agar, 5% Sheep blood agar, Chocolate agar and Mac Conkey agar. All cultures were incubated at 37°C under aerobic condition and addition to this blood agar and Chocolate agar also require 5-10% carbon dioxide atmosphere. Plates were evaluated for growth at 24 and 48 hours.

Endotracheal aspirate, bronchoalveolar lavage specimens were subjected to quantitative culture. All material is resuspended in the fluid and three serial dilutions are made (1/10, 1/1000, 1/100,000). These dilutions 0.01 ml is plated out in 5% Sheep Blood agar and incubated at 37°C under 5-10% carbon dioxide atmosphere for about 18-24 hours[6,49] .

The number of bacteria in the quantitative culture of ETA samples were expressed in colony-forming unit (cfu) per milliliter.(cfu/ml = number of colonies ×dilution factor ×inoculation factor) Quantitative threshold of organism is colony count of $\geq 10^5$ cfu/ml in ETAand $\geq 10^4$ cfu/ml BAL is consistent with pathogen and not a colonizer[8,50]. Bacterial isolates grown in culture were identified by means of Gram's staining and biochemical reactions by standard microbiological techniques[51]

.

Blood culture:

The inoculated blood culture bottles were incubated at 37°C and examined after 18 to 24 hours for any turbidity, discoloration or clotting. The first subculture was done onto Nutrient agar, blood agar and Macconkey agar plates and incubated aerobically at 37°C with 5-10% CO_2 for about 18-24 hours. These bottles were reincubated and checked for turbidity twice daily.

ANTIBIOTIC SUSCEPTIBILITY TESTING[52]

Antibiotic sensitivity testing was done on Mueller Hinton agar using Kirby Bauer disk diffusion method. Interpretation of the results was done by measuring the sizes of the zone of inhibition according to CLSI guidelines 2015(M-100-S25).

SEROLOGY-Antigen Tests[2,53,54,55]:

Legionella antigens in urine detects only serogroup1 (accounts for community acquired infection) with 90% sensitivity and 99% specificity.

Chlamydial antigen (LPSAg) can be demonstrated by ELISA or micro immunofluorescence method.

Pneumococcal urine antigen test is also quite sensitive and specific (80% and >90%, respectively).

MANAGEMENT OF PNEUMONIA[2,5]:

1.COMMUNITY ACQUIRED PNEUMONIA[56]:

Outpatients-

- without comorbidities: Azithromycin [500 mgPO once, then 250 mg qd] or Clarithromycin [500 mg PO bid] , Doxycycline (100 mg PO bid).

- with comorbidities: respiratory fluoroquinolone (levofloxacin [750 mg PO qd] moxifloxacin [400 mg PO qd], gemifloxacin [320 mg PO qd]) or β-lactam amoxicillin [1g tid] or amoxicillin/clavulanate [2g bid] or ceftriaxone [1–2 g IV qd] , cefuroxime [500 mg PO bid]) cefpodoxime [200 mg PO bid], with a macrolide.

Inpatients:

- **Non-ICU-** respiratory fluoroquinolone (levofloxacin [750 mg PO qd] moxifloxacin [400 mg PO qd] , gemifloxacin [320 mg PO qd]) β-lactam antibiotics (ampicillin [1–2 g IV q4–6h], cefotaxime [1–2 g IV q8h], ., ceftriaxone [1–2 g IV qd], ertapenem [1 g IV qd]) with a macrolide (oral clarithromycin or azithromycin or IV azithromycin[1 g once, then 500 mg qd])
- **ICU-** β-lactam antibiotics (ampicillin-sulbactam [2 g IV q8h], ceftriaxone [2 g IV qd], or cefotaxime [1–2 g IV q8h]) with either fluoroquinolone or azithromycin .

Special Consideration:

CA-MRSA: Add vancomycin (15 mg/kg q12h) or linezolid (600 mg IV q12h). *Pseudomonas aeruginosa*- antipseudomonal β-lactam antibiotics (Meropenem [1 g IVq8h]) , Imipenem [500 mg IV q6h], Piperacillin/tazobactam [4. 5 g IV q6h], Cefepime [1–2 g IV q12h], with either of fluoroquinolone {Levofloxacin (750 mg IV qd) or Ciprofloxacin (400 mg IV q12h)}or an aminoglycoside (Tobramycin [1. 7 mg/kg qd]) or Amikacin [15 mg/kg qd] .

2.NOSOCOMIAL PNEUMONIA[57]:

a.Patient without risk of MDR pathogen-

Ampicillin /sulbactam I.V. - 3g 6th hourly or Cefotaxime (q 6hr) /Ceftriaxone(q 24hr) -2g I.V or Moxifloxacin I.V.-400mg q24 hr or Ertapenem I.V.-1g q24 hr.

b.Patient with risk of MDR pathogen-

Linezolid (600 mg IV q12h) *or* Vancomycin (15 mg/kg q12h) for Gram positive bacteria along with β-lactam Ceftazidime (2 g IV q8h) or cefepime (2 g IV q8–12h) or Piperacillin/tazobactam (4. 5 g IV q6h) or Imipenem (500 mg IV q6h or 1 g IV q8h), or meropenem (1 g IV q8h) plus Ciprofloxacin (400 mg IV q8h) or levofloxacin (750 mg IV q24h), Gentamicin or tobramycin (7 mg/kg IV q24h) or amikacin (20 mg/kg IV q24h) for Gram-negative bacteria.

BACTERIAL RESISTANCE[58]:

Mechanism by which microrganism showing resistance to antibiotics are due to production of certain enzymes (β-lactamases) destroy active form of drug are produced by some microorganism, alteration in the drug permeability, alteration in the target site or metabolic enzymes responsible for drug action. Drug resistance may be non genetic (showing phenotypic resistance)or genetic(mutation in chromosome /plasmid mediated resistance) by origin.

BETALACTAMASES

This is a heterogeneous group of penicillin recognizing proteins. They belong to members of super family of active site serine protease. These enzymes inactivate β-lactam antibiotics (Penicillin, Cephalosporins).

CLASSIFICATION OF BETA LACTAMASES

Schemes of functional classification that were accepted by β-lactamase researchers include:

(i) In 1968, Cephalosporinases and penicillinases were grouped on the basis of reaction to specific antibody (Sawai et al).

(ii) In 1973, the Richmond and Sykes scheme classified the enzymes into five main divisions based on the substrate profile and the gene coding for β-lactamase.

(iii) In 1989, Bush scheme classified β-lactamase on the basis of molecular structure and the substrate inhibition.

(iv) In 1980, Ambler was the first to propose the Molecular structure classifications.

(v) More recently, Bush, Jacoby, and Medeiros devised a classification scheme based on the sequence of nucleotide on the genes for placing β-lactamases into functional groups and on the enzyme's biochemical properties and molecular structure[59].

(Classification schemes for bacterial b-lactamases)

Bush-Jacoby-Medeiros group	1989 Bush group	Richmond-Sykes class	Mitsuhashi-Inoue type	Molecular class	Preferred substrates	Inhibited by:		Representative enzyme
						CA[b]	EDTA	
1	1	Ia,Ib,Id	Csase[a]	C	Cephalosporins	-	-	AmpC from Gram negative bacteria, MIR-1
2a	2a	not included	Pcase V	A	Penicillin	+	-	Penicillinases from Gram Positive bacteria
2b	2b	III	Pcase I	A	Penicillins, Cephalosporins	+	-	TEM-1, TEM-2, SHV-1
2be	2b'	not included except K1 in class IV	Cxase	A	Penicillins, Narrow spectrum and extended spectrum Cephalosporins, Monobactams.	+	-	TEM-3 to TEM-26, SHV-2 to SHV-6, *Klebsiellaoxytoca* K1
2br	not included	not included	not included	A	Penicillins	±	-	TEM-30 to TEM-36, TRC-1
2c	2c	II,V	Pcase IV	A	Penicillins,Carbenicillins	+	-	PSE-1, PSE-3, PSE-4
2d	2d	V	Pcase II, Pcase III	D	Penicillins, Cloxacillin	±	-	OXA-1 to OXA-11, PSE-2 (OXA-10)
2e	2e	1c	Cxase	A	Cephalosporins	+	-	Inducible cephalosporinases from *Proteus vulgaris*
2f	not included	not included	not included	A	Penicillins, Cephalosporins, Carbapenams	+	-	NMC-A from *Enterobacter cloacae*, Sme-1 from *Serratia marcescens*
3	3	not included	not included	B	Mostβ lactams, including carbapenams	-	+	L1 from *Xanthomonasmaltophilia* CcrA from *Bacteroides Fragilis*
4	4	not included	not included	ND[c]	Penicillins	-		Penicillinase from *Pseudomonas Cepacia*

a Csase, cephalosporinase; PCase, penicillinase; CXase, cefuroxime-hydrolyzing b-lactamase.

b CA, clavulanic acid.

c ND, not determined.

EXTENDED SPECTRUM BETA LACTAMASES (ESBL)

ESBL are plasmid mediated betalactamases that produce resistance to broad spectrum betalactam antibiotics like 3rd and 4th generation cephalosporins, extended spectrum penicillin, Aztreonam[60].

METHODS FOR DETECTION OF EXTENDED SPECTRUM BETA LACTAMASES[61,62]

SCREENING OF ESBL-Disc Diffusion method[52,63]

The disc diffusion methods are the screening test for ESBL production by Escherichia coli, Klebsiella and Proteus mirabilis as proposed by CLSI 2015 guindelines use cefotaxime, ceftazidime, cefpodoxime, ceftriaxone and astreonam for the screening of ESBL production. Screening test is positive for ESBL is that the isolates showing resistant to 2 or more 3rd generation cephalosporin and it should be confirmed by phenotypic confirmatory test. The resistant zone size for ESBL as per CLSI 2015 guidelines**:** Cefotaxime ≤ 27mm, Ceftazidime ≤ 22mm, Ceftriaxone ≤ 27mm, Cefpodoxime ≤ 17mm and Aztreonam ≤ 27mm respectively.

CONFIRMATORY TEST FOR ESBL:

1. Phenotypic confirmatory disc diffusion test[11,52,64]

The CLSI advocates the phenotypic confirmatory test for the detection of production of ESBL by *Klebsiella* and *Escherichia coli* which use the cefotaxime or ceftazidime discs (30μg) with or without clavulanate (10μg). A Semiconfluent growth of test organism on Mueller Hinton agar shows difference of 5 mm along the cephalosporin with clavulanate disc compared to cephalosporin disc alone.

2.Minimum Inhibitory Concentration

- **Agar dilution method :**

Minimum inhibitory concentration was performed by agar dilution method Various concentration of third generation cephalosporins like cefotaxime or

ceftazidime 2µg to 2048µg/ml and cephalosporin with 4µg of clavulanic acid ranging from 0.5µg to 2048µg/ml of agar was tested with isolates. MIC is the least concentration at which there is no visible growth and it was obtained as eight fold decrease in CAZ-CL compared to ceftazidime.

- **Broth Micro dilution:**

Disc potentiation test can also be done using broth microdilution assays by using ceftazidime (0.25to128µg/ml), ceftazidime with clavulanate (0.25/4 to128/4 µg/ml), cefotaxime (0.25 to64µg/ml), and cefotaxime with clavulanate (0.25/4 to 64/4 µg/ml) decrease in MIC of two fold serial dilution of cephalosporin with clavulanate compared to the MIC of cephalosporin alone suggests positive for ESBL production.

COMMERCIAL METHODS AVAILABLE TO DETECT ESBL

(i) Epsilometer-Test for ESBLs[65,66]

Plastic drug impregnated strips are produced by AB bio disk in which one end contains a gradient of ceftazidime (MIC test ranges from 0.5µg - 32µg/ml) and with a ceftazidime gradient and constant concentration of clavulanate (4µg/ml). As per CLSI guidelines 2015, MIC value of ceftazidime – clavulanate should be ≥ 8 fold decrease in concentration than MIC value of ceftazidime alone.

(ii) Vitek ESBL[67]

Vitek ESBL cards contain cefotaxime and ceftazidme alone and cephalosporin plus constant concentration of clavulanate. Cards are inoculated in the same manner as that for regular vitek cards. Cards are analysed automatically as

soon as the growth in the control well has attained a set threshold. A prefixed reduction in the growth of cefotaxime and ceftazidime plus clavulanic acid containing wells is compared with the growth in the wells having cefotaxime/ceftazidime alone, indicates positive for ESBL producer. The sensitivity and specificity of the test is more than 90%.

GENOTYPIC METHOD TO DETECT ESBL[67,68,69]:

Detection of the common ESBL gene such as TEM, SHV and CTX-M by molecular method[10,68].

Test	**Advantages**	**Disadvantages**
DNA Probes	Specific for gene family(e.g., TEM or SHV)	Labour intensive, cannot distinguish between ESBLs and non ESBLs, and between variants of TEM or SHV
PCR	Easy to perform, specific for gene family(e.g.,TEM or SHV)	Require technical skill and expensive.
Oligotyping	Detects specific TEM variants	Requires specific oligonucleotide probes, labour intensive, cannot detect new variants.
PCR-RFLP	Easy to perform, can detect specific nucleotide changes	Nucleotide changes must result in altered restriction site for detection.
Nucleotide sequencing	Gold standard, can detect all variants	Labour intensive, can be technically challenging, can be difficult to interpret manually.
Real Time PCR	Rapid identification, minimum cross contamination	Expensive, technical skill required

METALLOBETALACTAMAES[70]:

Metallo-β-lactamases (MBL's) are carbapenemases produced mainly by Pseudomonas aeruginosa which require zinc at the site of action. They are designated in Ambler's Class B and Bush-Jacoby Medeiros Group 3. They hydrolyze virtually all β-lactam agents such as penicillin, cephalosporin, including the carbapenams.

Till now seven major types of MBL were described worldwide – IMP, SPM, VIM, GIM, SIM, AIM-1 and NDM-1. Among them, *bla*IMP and *bla*VIM are the most common types of MBLs that are prevalent worldwide. From India, only *bla*VIM and NDM-1 have been reported in *P. aeruginosa* in the past.

TESTS TO DETERMINE METALLO BETA LACTAMASES AMONG NON FERMENTERS

SCREENING FOR MBL

An isolate of *P. aeruginosa* was considered screen-test positive for MBL when it was Imipenem resistant: 10 μg(IPM) and/or Meropemem: 10 μg (MRP) and/or Ceftazidime: 30 μg (CAZ) . Antibiotic sensitivity was done by the Kirby-Bauer disc diffusion method as per CLSI recommendation.

CONFIRMATION OF MBL PRODUCTION

MODIFIED HODGE TEST[71]:

MHA plate is streaked with the ATCC *Escherichia coli* 25922 and an imipenem disc is placed in the centre. Imipenem resistant isolates are inoculated from the edge of the disc to the periphery of the plate. It is incubated overnight and

read. Imipenem hydrolyzing strains produce distortion on the zone whereas non hydrolyzing zones do not produce any effect.

Amp C BETALACTAMASES[72]

METHODS TO DETECT Amp C PRODUCTION[73]

All Enterobacteriaceae isolates were screened for Amp C betalactamases production by disk diffusion method.

SCREENING OF Amp C PRODUCTION[74,75]:

All isolates were screened for cefoxitin susceptibility and those which had a zone diameter of ≤ 18 mm were suspected to be AmpC producers.

Amp C DISK TEST[7,70,76] :

In a MHA plate, a lawn culture of *E. coli* ATCC 25922 was made. Sterile saline (20µl) was put on the sterile disks (6 mm) which was later inoculated with several colonies of test organism. Cefoxitin 30µg disk (almost touching) was placed on a fresh inoculated plate. The inoculated disk was then placed adjacent to cefoxitin disk and incubated overnight at 35°C. The inference was made as follows:

Flattening or indentation of the cefoxitin inhibition zone in the vicinity of the test disk was taken as positive. A negative test had an undistorted zone.

MANAGEMENT OF INFECTION WITH ESBL – PRODUCING ORGANISMS[60,77]

ESBL producers are treated with Piperacillin – Tazobactam ,Cefoperazone – sulbactam in case of mild infection ,whereas severe infection are treated with Carbapenems (Ertapenem, Meropenem, Imipenem).

Amp C producers are usually resistant to cephamycins and oxyimino-beta lactams but they are sensitive to carbapenams but diminished porin expression makes them resistant to carbapenam as well which can be treated with Polymixins, Tigecycline, Fosfomycin or Colistin.

METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS*[48,78]

Staphylococcus aureus is the most important human pathogen present in the external environment and in the anterior nares of 20- 40% of adults. It is also seen in the axillae, intertriginous skin folds, the perineum, and the vagina. It is responsible for mild infections to severe life threatening infections. Penicillin was the drug of choice for the treatment of serious *S.aureus* infections. The advent of penicillin resistance in the *S.aureus* was due to the acquisition of plasmid borne genetic elements coding for β lactamase production. Later, Penicillinase-resistant, semisynthetic penicillins such as oxacillin, methicillin was the drug of choice due to its incorrect use, MRSA showing difference in penicillin binding protein known as PBP2a from a chromosomal gene (mecA) has emerged. Initially1970s MRSA was accounting to 40-60% of all nosocomial infection but after 1990 it has been associated with population in the community who has no contact with hospital known as community- associated MRSA (CA-MRSA) strains which has caused death of 4 children due to necrotizing pneumonia[79]. MRSA prevalence in India has increased from 12% (1992) to 40% (2009)[12].

MECHANISM OF RESISTANCE

The chromosomally localized mecA gene responsible for methicillin resistance acts by synthesizing Penicillin binding protein 2a in turn downregulates the cross linking of peptidoglycan layer, through which it shows resistance to betalactam antibiotics. Four different SCC mec elements have been recognized. SCCmec type I, II and III is associated with Health care associated MRSA (HA-MRSA). Community associated MRSA tend to carry SCC Type IV element and Panton- Valentine leukocidin as virulence factor.

METHODS TO DETECT MRSA

PHENOTYPIC METHOD

1. Cefoxitin disc diffusion test[12,76]-

The test was performed by placing 30μg of Cefoxitin disc in the Mueller Hinton Agar plate inoculated with test organism. The plate was kept in incubator at a temperature of 37˚C. The zone of inhibition was determined after 24 hrs and the zone size was interpreted as Susceptible $\geq$ 22mm and Resistant $\leq$ 21 mm.

2. Oxacillin screen agar method[80]

Oxacillin screening is done by using 6 μg/ml of oxacillin in Mueller Hinton agar to confirm all methicillin resistant strains .The strains which grow in this medium is considered as methicillin resistant Staphylococcus aureus (MRSA).

3. MIC determination

(i) Agar dilution method

4-5 discrete colonies were emulsified onto 4-5 ml of nutrient broth which is adjusted to 0.5 McFarlands standard.0.0001ml is used as the final inoculum. The concentration of oxacillin used is 32µg-0.015µg/ml. After drying,1µl of inoculum is inoculated in the plates using a calibrated loop . The plates are incubated at 37˚C for 24hrs.MIC is the lowest concentration at which no visible growth occurs. Susceptible - ≤ 2µg/ml, Resistant - ≥ 4µg/ml

(ii) Broth dilution method

To a Mueller Hinton broth with 4% NaCl, serial dilution of oxacillin is added. Few colonies of S. aureus are emulsified into fresh peptone water and adjusted to match 0.5 McFarlands standard which is used as inoculum. It is incubated at 33-35c or 24 hrs. Oxacillin MIC <2µg/ml sensitive and Resistant> 2µg/ml.

(iii) E-TEST[12,52,81]:

MIC test should be performed to differentiate Vancomycin susceptible isolates of S.aureus from Vancomycin intermediate isolates. Plastic drug impregnated strips are produced by in which a gradient concentration of Vancomycin (MIC test ranges from 0.016µg - 256µg/ml) was applied on a MHA plate on which MRSA isolates were swabbed and the plates were incubated at 37ºC overnight. Interpretation of Vancomycin MIC E-test for MRSA as per CLSI guidelines 2015 are as follows [52]: <2 µg/ml is sensitive,4-8 µg/ml is Intermediate and ≥16 µg/ml is Resistant.

GENOTYPIC METHODS [82]

Multiplex PCR for MRSA detection of mec A and fem B genes, coag genes, ccr genes, nuc genes, toxin genes.

1. Pulsed Field Gel Electrophoresis

From an overnight grown culture of a single colony, a bacterial pellet is processed and the restriction fragments are separated on the gel. Gel is stained with ethidium bromide. The photo is taken under Ultra Violet light. Strain relatedness among CA-MRSA and HA-MRSA isolates can be investigated.

2. Real time PCR

MRSA isolates are detected directly from blood culture bottles using real time PCR assays. Based on melting curve analysis, the assay differentiates into clusters.

3. **Multi locus Sequence Typing (MLST)**

The clonal evaluation of MRSA is detected by MLST. Sequential analysis from 7 Staphylococcus aureus shows the housekeeping genes as follows i.e., aroE, arcC, glpF, gmK, pt, tpi and yqil. Each isolate is defined by all the alleles of the seven genes. This results in an allele profile / gene sequence type (ST).

4. Microarray Analysis

Multiplex PCR products can be used as hybridization samples. After hybridization at the test site of the microarray, detection of fluorescence is done automatically by the instrument images of the array. Automatically captured image is then analysed using the image analysis software of the instrument.

Management of MRSA infections[60, 77]

The drug of choice for serious infections caused by *Methicillin Resistant Staphylococcus aureus* is glycopeptide antibiotics (Vancomycin or Teicoplanin).

PREVENTION:

VACCINATION[2, 31, 83, 84, 85] :

Vaccination against influenza, pneumococcus, *Haemophilus influenza b* is used to reduce the burden of pneumonia.

1. Influenza vaccines are of two types intranasal live-attenuated cold-adapted vaccine (not indicated in immunocompromised patients) and intramuscular inactivated vaccine. During an influenza outbreak, vaccination given immediately with chemoprophylaxis (zanamivir or oseltamivir for 2 weeks).Efficacy is about it prevents 53% pneumonia, 50% from hospitalization and 68% from death.
2. Pneumococcus- Pneumococcal infection severe due to its invasive property and drug resistance. two types of pneumococcal vaccine - PPV23 pneumococcal polysaccharide vaccine contains capsular material from 23 pneumococcal serotypes and PCV13, protein conjugate pneumococcal vaccine contain capsular polysaccharide from 13 protein commonly affecting children. PCV13has an immunogenic protein which induces the production of T cell–dependent antigens for long-term immunologic memory. PCV13 vaccine is given for children, immunocompromised patients and elderly. VAP-oral care, hand hygiene, use of prophylactic agent of gastric ulcer, gloves usage, protocol directed weaning procedure, change of humidifier weekly and suction system for each patient[9].

MATERIALS AND METHODOLOGY

The study of bacterial isolates causing Pneumonia in 205 adult patients with suspected Pneumonia includes 150 Community Acquired Pneumonia (CAP), 30 Hospital Acquired Pneumonia (HAP) and 25 Ventilator Associated Pneumonia (VAP).

STUDY DESIGN : Cross sectional study

STUDY PERIOD : January 2015 to June 2016

STUDY PLACE : Government Kilpauk Medical College and Hospital, Chennai.

INCLUSION CRITERIA

1. Clinically suspected and radiologically proven cases of pneumonia.
2. Patient above 18 years of age (adult).

EXCLUSION CRITERIA:

1. Patient with active tuberculous leision was excluded.
2. Patient taking antibiotics currently and past for the period of about two weeks were excluded.

SAMPLE COLLECTION, TRANSPORT AND PROCESSING:

Totally 205 respiratory samples from Pneumonia in adult patients attending Government Kilpauk Medical College and Hospital includes 150 sputum samples from Community Acquired Pneumonia(CAP), 30 sputum samples from Hospital Acquired Pneumonia(HAP) and 25 Endotracheal samples from Ventilator Associated Pneumonia(VAP).

Blood samples was collected from pneumonia patients having symptoms of fever (Temp ≥37.8ºC)[86] were included 80 samples from CAP, 12 samples from HAP and 25 samples from VAP.

After obtaining informed consent from these patients respiratory samples and blood were collected under sterile precaution and transported immediately to the laboratory in appropriate settings and sample processing done.

SAMPLE COLLECTION [46, 87]:

1. **Sputum – expectorated or induced[41,3,28]**

Deeply coughed out or when the sputum is scanty it was induced with saline nebulisation and was collected in a disposable leak proof sterile ,wide mouthed container with tight fitting lid after giving proper instruction to the patient.

2. **Endo Tracheal aspirate (ETA)[6,45]**

Endo Tracheal aspirate (ETA) was collected from the patients who have been in mechanical ventilation for more than fourty eight hours with suspected VAP. One milliliter of ETA was collected in a sterile screw capped plastic container by using twenty two inch Ramson's 12 F suction catheter with a mucus extractor which was introduced slowly in to the endotracheal tube for about a distance of twenty five to twenty six centimeter.

3. **Blood[86]:**

Blood culture was performed in all cases of suspected Pneumonia with fever (temp.≥37.8ºC) prior to starting antibiotics. Under aseptic precaution, 5 ml of blood was collected by venipuncture using sterile syringe and transferred after removing the needle into the blood culture bottle containing 50 ml (1:10

dilution)[45]of Brain heart infusion broth. Samples collected were sent immediately to Microbiology laboratory without delay.

SAMPLE PROCESSING

a) Macroscopic examination[41]:

The sputum was examined for colour (rusty, red currant jelly was noted), consistency, purulent/non purulent to distinguish it from saliva.

b) Direct Microscopy:

The Sputum, Endotracheal aspirate specimens were subjected to the following microscopic examination using standard laboratory techniques. Gram staining was done and examined for the presence of relative number of polymorphonuclear cells and squamous epithelial cells.

Criteria for assessing the quality of respiratory samples[48]:

Bartlett's grading:

Number of neutrophil (lpf)	**Grade**
<10	0
10 - 25	+1
>25	+2
Presence of mucus	+1

Number of epithelial cells	**Grade**
10 - 25	-1
>25	-2

Total number of polymorphonuclear cells and epithelial cells and in 20-30 LPFs was calculated and averaged the total score was arrived. A final score of 0 or less indicated lack of active inflammation or contamination, and a score of 1 and above was considered an acceptable sample. Presence of >25 PMN cells with less

than ten squamous epithelial cells per low power field in Gram stained smear of ETA samples was accepted for culture [6].

CULTURE [4]:

Sputum culture:

Sputum samples were then plated into the following agar media: Nutrient agar 5% Sheep blood agar, Chocolate agar and MacConkey agar. All cultures were incubated at 37°C under aerobic condition and addition to this blood agar and Chocolate agar plates were kept under 5-10% carbon dioxide atmosphere. Plates were evaluated for growth at 24 and 48hours.

Endotracheal aspirate culture:

Endotracheal aspirate specimens were subjected to quantitative culture [6,88,89]. Colony count of $\geq 10^5$ cfu/ml was consistent with pathogen and not a colonizer[7,8].

Endotracheal aspirate sample was resuspended in the fluid and three serial dilutions were made (1/10, 1/100, 1/1000). Of these 0.01 ml from 1/1000 dilutions was plated on to Blood agar. The number of bacteria in culture of ETA samples were expressed in colony-forming unit (cfu) per milliliter.(cfu/ml = number of colonies ×dilution factor ×inoculation factor)Presence of single colony in 0.01ml of 1/1000 dilution indicate $\geq 10^5$ colonies[49] . Bacterial isolates grown in culture were identified by means of Gram's staining and biochemical reactions by standard microbiological techniques.

Blood culture[45,48]:

The inoculated blood culture bottles were incubated at 37°C and examined after 18 to 24 hours for any turbidity, discoloration or clotting. The first subculture

was done onto Nutrient agar, blood agar and Macconkey agar plates and incubated at 37°C for 18 to 24 hours, meanwhile in addition to these blood agar plate was kept under 5-10%CO_2. These bottles were reincubated and checked for turbidity twice daily.

ANTIBIOTIC SUSCEPTIBILITY TESTING[52]

Antibiotic sensitivity testing was done on Mueller Hinton agar using Kirby Bauer disk diffusion method. Interpretation of the results was done by measuring the sizes of the zone of inhibition according to CLSI guidelines 2015(M-100-S25).Quality control strains used are as follows[7]:ATCC 25922 *Escherichia coli* , ATCC 27853 *Pseudomonas aeruginosa and* ATCC 25923 *Staphylococcus aureus* .

DETECTION OF EXTENDED SPECTRUM BETA LACTAMASES

All Enterobacteriaceae isolates were screened for betalactamases production by disk diffusion method and confirmed by phenotypic confirmatory disc diffusion test.

Disk diffusion methods-screening for ESBL[52]:

Disk diffusion test was done for all Enterobacteriaceae isolates against Cefotaxime (30 μg), Ceftriaxone (30 μg), and Ceftazidime (30 μg) antibiotic disks for the screening of the isolates for potential ESBL production.

Overnight incubation was done at 37˚C after which the zone size was read as per CLSI recommendations for ESBL screening criteria in which the isolates showed resistant to two or more 3rd generation Cephalosporins[90].

Antibiotics	Zone of inhibition – interpretation
Cefotaxime (30µg)	≤27mm
Ceftriaxone(30µg)	≤25mm
Ceftazidime(30µg)	≤22mm

Quality controls were performed using

ATCC 700603 *Klebsiella pneumoniae* - Positive control.

Phenotypic confirmatory disc diffusion test[11,52]

This test was done for all Enterobacteriaceae isolates against Ceftazidime (30 µg) antibiotic discs with and without clavulanic acid (10 µg). These discs were placed on a Mueller –Hinton agar plate inoculated with bacterial suspension equivalent to 0.5 McFarland standards. Overnight incubation was done at 37°C after which the result was interpreted as follows:

If the zone diameter of Ceftazidime with clavulanic acid was increased ≥ 5 mm when compared with Ceftazidime alone was taken as positive for ESBL production.

MIC determination – E Test method[65]

Minimum inhibitory concentration was calculated for all isolates of ESBL by Epsilometer test**.**

Epsilometer-Test for ESBL [10,52]

Plastic drug impregnated strips are produced by Himedia in which one end contains a gradient of ceftazidime (MIC test ranges from 0.5µg - 32µg/ml) and Ceftazidime+clavulanic acid (MIC test ranges from 0.064 µg - 4µg/ml) on the other end was applied on a MHA plate on which ESBL isolates are swabbed. The plates were incubated at 37ºC overnight.

As per CLSI guidelines 2015, MIC value of ceftazidime – clavulanate should be ≥ 8 fold decrease in concentration than MIC value of ceftazidime alone. (Manufacturer recommends MIC value in the ratio of CAZ:CAC ≥ 8).

DETECTION OF ESBL PRODUCERS BY POLYMERASE CHAIN REACTION (PCR) [10]

DNA Extraction methods

DNA extraction was done with the help of DNA Purification kit (PureFast Bacterial Genomic DNA purification kit) and polymerase chain reaction master mix.

Constituents of Master Mix 2X

Taq DNA Polymerase - 2Units.

10X Taq reaction buffer

2mM Magnesium Chloride.

10mMdNTPs mix - 1µl.

Polymerase Chain Reaction additives.

Agarose for the purpose of Gel Electrophoresis - Agarose, 50XTAE buffer, 6Xgel loading buffer, Ethidium bromide were used.

PRIMERS

CTX-M primer(Product size - 269bp)

5'-TTATGCGCAGACGAGTGCGGTG-3'

5'-TCACCGCGATAAAGCACCTGCG-3'

SHV primer(Product size- 276bp)

5'-CGCCGCCATTACCATGAGCGAT-3'

5'-ACCCGATCGTCCACCATCCACT-3'

TEM primer(Product size - 250bp)

5’-CCAAACGACGAGCGTGACACCA-3’

5’-AGCGCAGAAGTGGTCCTGCAAC-3’

Procedure of DNA Extraction

One ml of overnight culture of *Klebsiella pneumoniae* was centrifuged at 6000 rpm for five minutes and supernatant was discarded.Pellet was suspended in 200µl of Phosphate Buffer Saline (PBS).

To the suspension twenty microlitre of lysozyme(10mg/ml) , 180µl of lysozyme digestion buffer was added and incubated at 37ºC for fifteen minutes. Mixing with 400µl of binding buffer ,5 µl of internal control template and 20 µl of Protienase K was done by inverting the tube several times and then incubated at 56ºC for 15 minutes.

300µl of ethanol was added and mixed well. Whole lysate was transferred into Pure Fast spin column. It was then centrifuged for one minute at 10000 rpm. Discard flow through and 500µl of Wash Buffer-1 was added and centrifuged at 10000 rpm for 1Minute. Discard flow through and 500µl of Wash Buffer-2 was added and centrifuged at10000 rpm for 1minute. This procedure was done for two times.

Discard flow through centrifuged column for 1 more minutes so that any residual ethanol will be removed. The content in spin column was transferred to a 1.5 ml micro-centrifuge tube. 100µl of Elution Buffer was added to elute the DNA, and centrifuged for 2 minute.

Procedure of Polymerase Chain Reaction

1. Reaction was done with the components in PCR vial-10µl of Master Mix, 5µl Genomic DNA, 5µl Primer mix which constitutes about 20 ml of total volume.
2. All these were mixed gently and spinned down briefly.
3. They were then placed in the PCR machine and programmed;

   Initial Denaturation: 95ºC for 5 minutes

   Denaturation: 94ºC for 30seconds in cycles of 35

   Annealing: 58ºC for 30 seconds in cycles of 35

   Extension: 72ºC for 30 seconds in cycles of 35

   Final extension: 72º C for 5 minutes

Method to perform Agarose gel electrophoresis:

- 2gm agarose was mixed in 100ml of 1X TAE buffer; it was melted by heating in a micro oven.
- When the temperature of agarose gel was about 60ºC, Ethidium bromide (5µl) was added.
- Into the gel platform warm agarose solution was added slowly.
- Till the agarose got solidified it was kept undisturbed.
- Into the submarine gel tank1XTAE buffer was added.
- Gel platform was kept into tank without any disturbance. The tank buffer level

  was kept maintaining upto 0.5cm above the gel.
- Polymerase Chain Reaction samples were loaded after mixing with gel loading

  dye and 10µl HELINI 100bp DNA Ladder.
- Electrophoresis was done at 50V till the dye reaches 3/4th distance of the agarose gel. The Agarose gel was seen with UV Transilluminator, the pattern of the bands were seen and interpreted.

DETECTION OF METALLOBETALACTAMASE PRODUCERS[7]:

Meropenem: 10µg (MRP) (Zone of inhibition ≤18mm) resistant *Pseudomonas aeruginosa* isolates were taken for screening MBL production. Antibiotic sensitivity testing was done by the Kirby-Bauer disc diffusion method.

METHODS TO DETECT AmpC PRODUCTION[73]

All Enterobacteriaceae isolates were screened for AmpC betalactamases production by disk diffusion method.

SCREENING OF AmpC PRODUCTION:

All isolates were screened for cefoxitin (30µg) susceptibility and those which had a zone diameter of ≤ 18 mm were suspected to be AmpC producers[74].

AmpC DISK TEST[7,6,70,76] :

In a MHA plate, a lawn culture of *E. coli* ATCC 25922 was made. Sterile saline (20µl) was put on the sterile disks (6 mm) which was later inoculated with several colonies of test organism. Cefoxitin30µg disk (almost touching) was placed on a fresh inoculated plate. The inoculated disk was then placed adjacent to cefoxitin disk and incubated overnight at 35°C. The inference was made as follows: Flattening or indentation of the cefoxitin inhibition zone in the vicinity of the test disk was taken as positive. A negative test had an undistorted zone.

TESTS TO DETECT METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS(MRSA)*[12]

All isolates of *Staphylococcus aureus* were screened for MRSA by disk diffusion method and confirmation done by genotypic method.

CEFOXITIN DISC DIFFUSION TEST

The test was performed by placing 30µg of Cefoxitin disc in the Mueller Hinton Agar plate inoculated with test organism. The plate was kept in incubator at a temperature of 37˚C. The zone of inhibition was determined after 24 hrs and the zone size was interpreted as Susceptible ≥ 22mm & Resistant ≤ 21 mm.

VANCOMYCIN E-TEST[12,52]:

MIC test was performed to differentiate Vancomycin susceptible isolates of *S.aureus* from Vancomycin intermediate isolates. Plastic drug impregnated strips are produced by in which a gradient concentration of Vancomycin (MIC test ranges from 0.016µg - 256µg/ml) was applied on a MHA plate on which MRSA isolates were swabbed and the plates were incubated at 37ºC overnight. Interpretation of Vancomycin MIC E-test for MRSA as per CLSI guidelines 2015 are as follows[52]:

Vancomycin concentration	**Interpretation**
< 2 µg/ml	Sensitive
4-8 µg/ml	Intermediate
≥16 µg/ml	Resistant

Detection of mec A gene in MRSA isolates by polymerase chain reaction (PCR)

DNA Extraction methods

DNA extraction was done with the help of DNA Purification kit (PureFast Bacterial Genomic DNA purification kit) and polymerase chain reaction master mix. Constituents of Master Mix 2X -10X Taq reaction buffer,Taq DNA Polymerase - 2Units, 10mMdNTPs mix - 1µl , 2mM Magnesium Chloride. 10mMdNTPs mix - 1µl.

Agarose for the purpose of Gel Electrophoresis - Agarose, 50XTAE buffer,6Xgel loading buffer, Ethidium bromide were used. mec A gene primer mix-5µl/reaction .PCR product – 220bp.

Forward primer 5′-TGGCTATCGTGTCACAATCG-3′

Reverse primer 5′-CTGGAACTTGTTGAGCAGAG-3′

Procedure of DNA Extraction

One ml of overnight culture of Staphylococcus aureus was centrifuged at 6000 rpm for five minutes and supernatant was discarded. Pellet was suspended in 200µl of Phosphate Buffer Saline (PBS).

To the suspension twenty microlitre of lysozyme(10mg/ml) , 180µl of lysozyme digestion buffer was added and incubated at 37ºC for fifteen minutes. Mixing with 400µl of binding buffer ,5 µl of internal control template and 20 µl of Protienase K was done by inverting the tube several times and then incubated at 56ºC for 15 minutes. 300µl of ethanol was added and mixed well. Whole lysate was

transferred into Pure Fast spin column. It was then centrifuged for one minute at 10000 rpm. Discard flow through and 500µl of Wash Buffer-1 was added and centrifuged at 10000 rpm for 1Minute.Discard flow through and 500µl of Wash Buffer-2 was added and centrifuged at10000 rpm for 1minute. This procedure was done for two times.

Discard flow through centrifuged column for 1 more minutes so that any residual ethanol will be removed. The content in spin column was transferred to a 1.5 ml micro-centrifuge tube.100µl of Elution Buffer was added to elute the DNA, and centrifuged for 2 minutes.

Procedure of Polymerase Chain Reaction

1. Reaction was done with the components in PCR vial-10µl of Master Mix, 5µl Genomic DNA, 5µl Primer mix which constitutes about 20 ml of total volume.
2. All these were mixed gently and spinned down briefly.
3. They were then placed in the PCR machine and programmed;

   Initial Denaturation: 95ºC for 5 minutes

   Denaturation: 94ºC for 30seconds in cycles of 35

   Annealing: 58ºC for 30 seconds in cycles of 35

   Extension: 72ºC for 30 seconds in cycles of 35

   Final extension: 72º C for 5 minutes

Method to perform Agarose gel electrophoresis:

- 2 gm agarose was mixed in 100ml of 1X TAE buffer; it was melted by heating in a micro oven.
- When the temperature of agarose gel was about 60ºC, Ethidium bromide (5µl) was added.
- Into the gel platform warm agarose solution was added slowly.
- Till the agarose got solidified it was kept undisturbed.
- Into the submarine gel tank1XTAE buffer was added.
- Gel platform was kept into tank without any disturbance. The tank buffer level was kept maintaining upto 0.5cm above the gel.
- Polymerase Chain Reaction samples were loaded after mixing with gel loading dye and 10µl HELINI 100bp DNA Ladder.
- Electrophoresis was done at 50V till the dye reaches 3/4th distance of the agarose gel. The Agarose gel was seen with UV Transilluminator, the pattern of the bands were seen and interpreted.

STATISTICAL ANALYSIS

The test outcome was observed, recorded and analysed. The data that were analysed and presented in the form of statistical tables, pie charts and histograms if necessary in appropriate places. P values were calculated by Chi – Square test to compare the proportion between categorical variables. If expected

cell frequency is less than five in more than 20% of cells then Fisher's exact Chi – Square test is applied. SPSS (Stastical package for the social science) version 22.0 is used to analyse the data. Significance level is fixed as 5% (α=0.005). The significant findings was further discussed in detail and compared with other similar studies published in reputed scientific journals. The clinical application of these findings will be stressed for better patient care.

RESULTS

Patients with clinical symptoms and radiological evidence of pneumonia attending medicine OPD and those admitted in IMCU and other wards at Government Kilpauk Medical College and Hospital, Chennai were studied for the profile of microorganisms isolated by culture with their Antibiotic sensitivity pattern. Resistant isolates were studied for Extended Spectrum BetaLactamases (ESBL), Metallobetalactamases (MBL), Amp C betalactamases and Methicillin Resistant *Staphylococcus aureus* (MRSA) . The study was done between January 2015 to June 2016. 205 patients with pneumonia, of which patients with Community acquired pneumonia (CAP) (150), Hospital acquired pneumonia (HAP) (30) and Ventilator associated pneumonia (VAP) (25) were studied. The observations were recorded and analysed. The results were as follows:

TABLE NO: 1 AGE WISE DISTRIBUTION IN PNEUMONIA (n=205).

AGE (YEARS)	**CAP (n=150)**	**HAP (n=30)**	**VAP (n=25)**
20-40	27(18%)	7(23.33%)	2(8%)
40-60	70(46.66%)	10(33.33%)	14(56%)
>60	53(35.33%)	13(43.33%)	9(36%)

As per Table no.1, Patients in the age group of 40-60 years were highly affected by CAP and VAP showing CAP 70(46.66%) and VAP 14(56%). In HAP, patients in the age group of above 60 years were mostly affected with 13(43.33%).

TABLE NO: 2 – GENDER WISE DISTRIBUTION IN PNEUMONIA (n=205).

GENDER	**CAP(n=150)**	**HAP(n=30)**	**VAP(n=25)**
Males	102(68%)	20(66.66%)	16(64%)
Females	48(32%)	10(33.33%)	9(36%)

As per Table no.2, adult males were more commonly affected by pneumonia than Females, CAP 102(68%), HAP 20 (66.66%) and VAP 16 (64%).

TABLE NO: 3 – RISK FACTORS OF PATIENTS WITH PNEUMONIA(n=205)

Risk Factors	CAP **(n=150)**	HAP**(n=30)**	VAP**(n=25)**
Smoking	73(48.66%)	16(53.33%)	18(72%)
Diabetes mellitus	58(38.66%)	14(46.66%)	14(56%)
Chronic obstructive airway disease	33(22%)	8(26.66%)	6(24%)
Alcoholism	28(18.66%)	13(43.33%)	13(52%)

As per Table no.3, Smoking is the most common risk factor in CAP 73(48.66%) followed by diabetes, COPD and alcoholism

TABLE NO: 4 - SYMPTOMS OF PATIENTS WITH CAP AND HAP

Symptoms	**CAP(n=150)**	**HAP(n=30)**
Cough	142(94.66%)	26(86.66%)
Fever (temp >37.8ºC)	80(53.33%)	12(40%)
Breathlessness	53(35.33%)	14(46.66%)
Chest pain	28(18.66%)	9(30%)

As per Table no.4, Cough was the most common presenting symptom of pneumonia, 142(94.66%) in CAP and 26(86.66%) in HAP followed by fever, breathlessness and chest pain.

TABLE NO: 5

CHEST XRAY PATTERN OF PATIENTS WITH PNEUMONIA (n=205)

LUNG LOBES AFFECTED	**CAP (n=150)**	**HAP (n=30)**	**VAP (n=25)**
Right lower lobe	54 (36%)	10 (33.33%)	9 (36%)
Right middle lobe	26 (17.33%)	6 (20%)	4 (16%)
Left lower lobe	23 (15.33%)	3 (10%)	3 (12%)
Both lobes	18 (12%)	8 (26.66%)	6 (24%)
Right upper lobe	16 (10.6%)	2 (6.66%)	2 (8%)
Left upper lobe	13 (8.6%)	1 (3.33%)	1 (4%)

As per Table no.5, Right lower lobe was the most commonly involved lung lobe seen in Chest radiography of pneumonia patients with 54(36%) in CAP, 10(33.33%) in HAP and 9(36%) in VAP.

TABLE NO: 6 – SAMPLES COLLECTED FROM PNEUMONIA PATIENTS AND THEIR CULTURE GROWTH

PNEUMONIA	SAMPLES	CULTURE POSITIVE	CULTURE NEGATIVE
CAP	Sputum(n=150)	83(55.33%)	67(44.66%)
	Blood(n=80)	8(10%)	72(90%)
HAP	Sputum(n=30)	26(86.66%)	4(13.33%)
	Blood(n=12)	2(16.66%)	10(83.33%)
VAP	Endotracheal aspirate(n=25)	25(100%)	0
	Blood(n=25)	5(20%)	20(80%)

As per Table no.6, Respiratory samples collected from pneumonia patients showed growth in culture as follows: 83(55.33%) in CAP, 26(86.66%) in HAP and 25(100%) in VAP. P value is <0.001 and is statistically significant.

Blood samples collected from pneumonia patients showed growth in culture as follows: 8(10%) in CAP, 2(16.66%) in HAP and 5(20%) in VAP.

CHART 1
GENDER DISTRIBUTION IN PNEUMONIA (n=205)

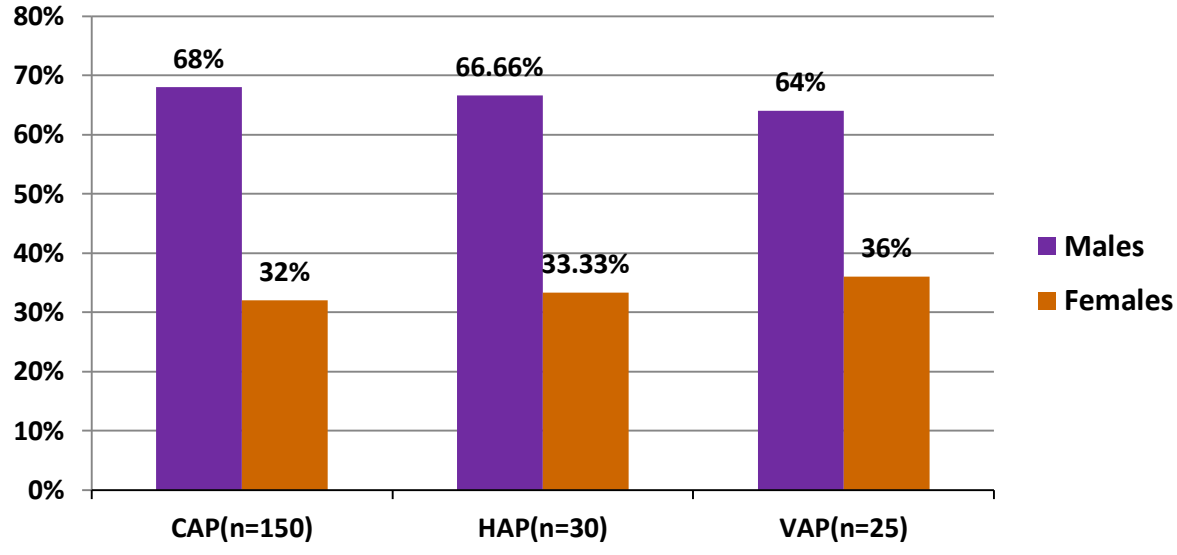


CHART 2
CULTURE POSITIVE IN RESPIRATORY SAMPLES OF PNEUMONIA

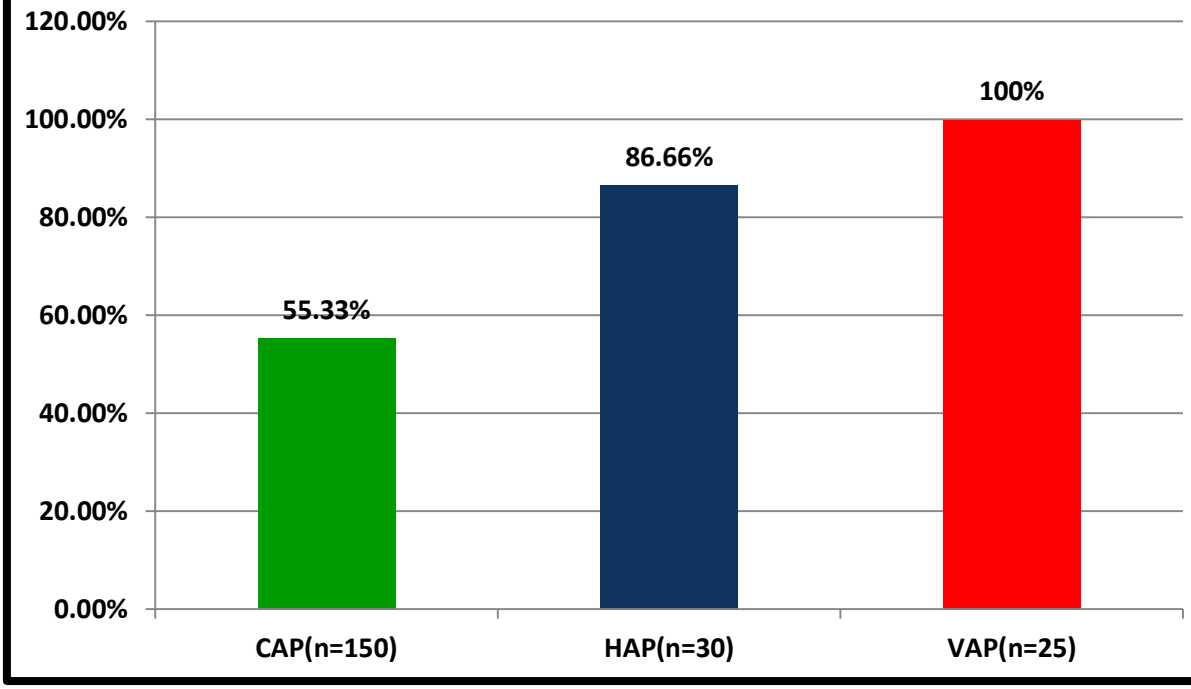


TABLE NO 7 - DIRECT SMEAR vs. CULTURE IN RESPIRATORY SAMPLES OF PNEUMONIA PATIENTS

CAP (n=150)

CULTURE	Direct Smear Positive	Direct Smear Negative
Culture Positive	80(53.33%)	3(2%)
Culture Negative	0	67(44.66%)

HAP (n=30)

CULTURE	Direct Smear Positive	Direct Smear Negative
Culture Positive	24(80%)	2(6.66%)
Culture Negative	0	4(13.33%)

VAP (n=25)

CULTURE	Direct Smear Positive	Direct Smear Negative
Culture Positive	21(84%)	4(16%)
Culture Negative	0	0

As per Table no.7, Respiratory samples with direct smear positive showing culture positivity are CAP 80(53.33%), HAP 24(80%) and VAP 21(84%). CAP 3(2%), HAP 2(6.66%) and VAP 4(16%) showed direct smear negative but culture was positive.

TABLE NO: 8 - PURE Vs. MIXED GROWTH IN CULTURE

ORGANISM	CAP (n=83)	HAP (n=26)	VAP (n=25)
Monomicrobial	71(85.54%)	20(76.92%)	24(96%)
Polymicrobial	12(14.45%)	6(23.07%)	1(4%)

As per Table no.8, Culture growth from respiratory samples of pneumonia patients showed both Monomicrobial and Polymicrobial growth.

TABLE NO 9- A: GRAM POSITIVE Vs. GRAM NEGATIVE IN CULTURE OF RESPIRATORY SAMPLES OF PNEUMONIA PATIENTS

PNEUMONIA	SAMPLES	GRAM NEGATIVE BACILLI	GRAM POSITIVE COCCI	TOTAL
CAP	Sputum	68(71.57%)	27(28.42%)	95
HAP	Sputum	24(75%)	8(25%)	32
VAP	Endotracheal aspirate	20(76.92%)	6(23.07%)	26

As per Table no. 9- A , Among the organisms isolated from respiratory samples of pneumonia patients , Gram negative bacilli is the predominant isolate with 68(71.57%) from CAP, 24(75%) from HAP and 20(76.92%) from VAP.

TABLE NO 9- B: - GRAM POSITIVE Vs. GRAM NEGATIVE IN BLOOD CULTURE OF PNEUMONIA PATIENTS

PNEUMONIA	GRAM NEGATIVE BACILLI	GRAM POSITIVE COCCI	TOTAL
CAP	5(62.50%)	3(37.50%)	8
HAP	1(50%)	1(50%)	2
VAP	4(80%)	1(20%)	5

As per Table no. 9- B, Among the organisms isolated from blood samples of pneumonia patients , Gram negative bacilli is the predominant isolate in CAP 5(62.50%) and VAP 4(80%).

TABLE NO: 10- DISTRIBUTION OF ORGANISMS IN RESPIRATORY SAMPLES OF PNEUMONIA PATIENTS.

ORGANISMS	CAP	HAP	VAP	TOTAL
	SPUTUM(n=95)	SPUTUM(n=32)	ETA(n=26)	
Klebsiella pneumoniae	42 (44.21%)	12 (37.50%)	6(23.07%)	60
Staphylococcus aureus	19(20%)	4(12.5%)	6(23.07%)	29
Pseudomonas aeruginosa	12(12.63%)	3(9.37%)	5(19.23%)	20
Escherichia coli	10(10.52%)	5(15.62%)	4(15.38%)	19
Acinetobacter baumanii	2(2.10%)	2(6.25%)	5(19.23%)	9
CONS	6(6.31%)	2(6.25%)	0	8
Serratia marscesens	2(2.10%)	1(3.12%)	0	3
Streptococcus pneumoniae	2(2.10%)	0	0	2
Enterococcus faecalis	0	2(6.25%)	0	2
Proteus mirabilis	0	1(3.12%)	0	1

As per Table no.10, *Klebsiella pneumoniae* is the predominant organism grown in respiratory samples of pneumonia patients and their distribution are as follows: 42 (44.21%) in CAP, 12 (37.50%) in HAP, 6(23.07%) in VAP is both

CHART 3 : DISTRIBUTION OF ORGANISMS IN CAP (n = 95)

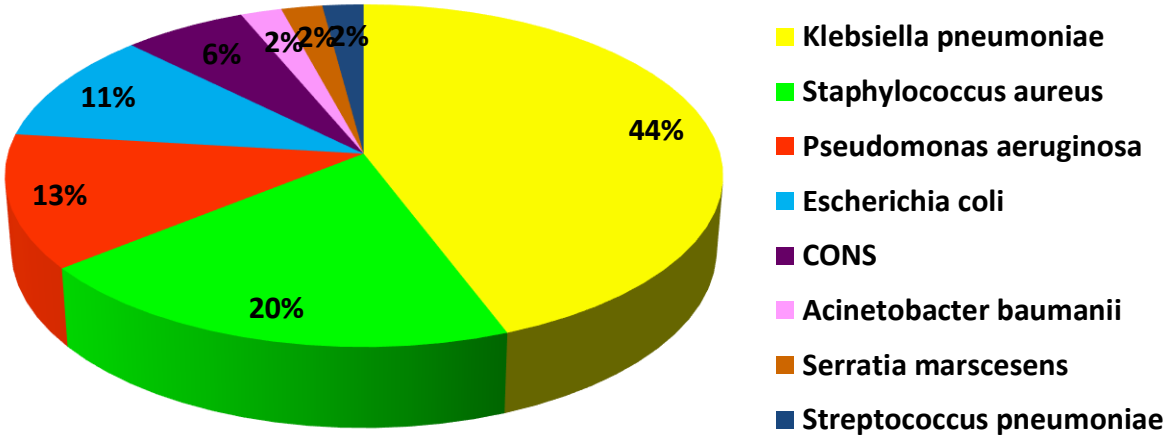


CHART 4 : DISTRIBUTION OF ORGANISMS IN HAP (n = 32)

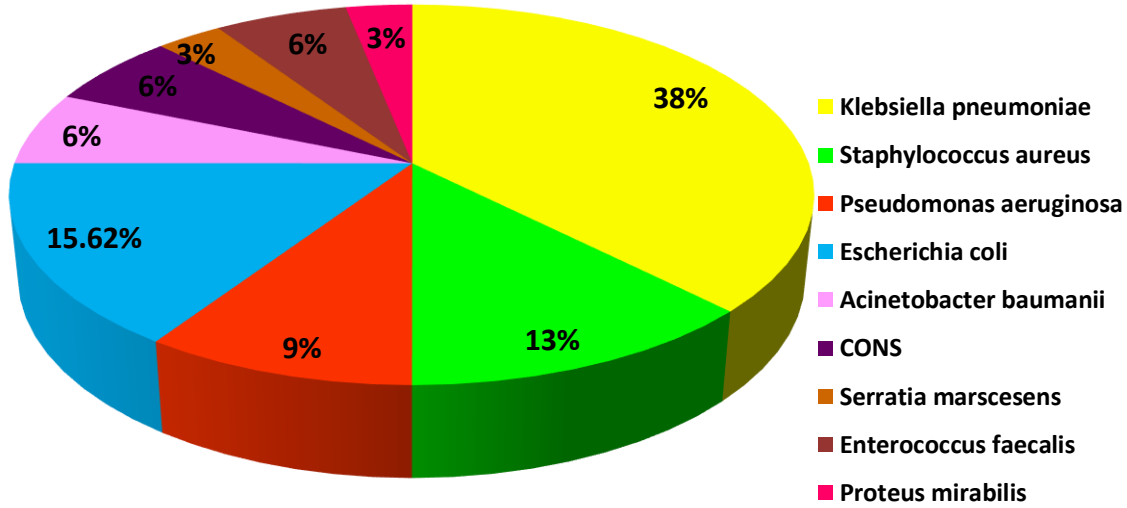
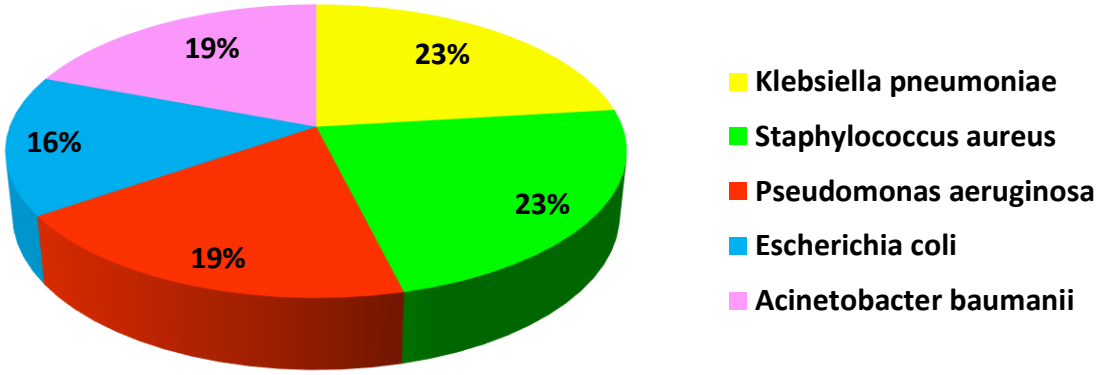


CHART 5 : DISTRIBUTION OF ORGANISMS IN VAP (n = 26)



Klebsiella pneumonia and Staphylococcus aureus . pvalue <0.035 and is stastically significant.Among non fermenters, *Pseudomonas aeruginosaand Acinetobacter baumanii* were grown in culture. *Staphylococcus aureus* is the predominant Gram positive cocci isolated in culture.

TABLE NO: 11- DISTRIBUTION OF ORGANISMS IN BLOOD SAMPLES OF PNEUMONIA PATIENTS.

ORGANISMS	**CAP(N=8)**	**HAP(N=2)**	**VAP(N=5)**	**TOTAL**
Klebsiella pneumonia	4 (50%)	1(50%)	3 (60%)	8
Staphylococcus aureus	2(25%)	1(50%)	1(20%)	4
Pseudomonas aeruginosa	1(12.50%)	0	1(20%)	2
CONS	1(12.50%)	0	0(20%)	1

As per Table no.11, *Klebsiella pneumoniae* is the Predominant organism grown in blood samples of pneumonia patients and their distribution are as follows: 4 (50%) in CAP and 3(60%) in VAP.

TABLE NO: 12 –A: ANTIBIOTIC SENSITIVITY OF GRAM NEGATIVE ISOLATES FROM SPUTUM SAMPLES OF CAP.

Antibiotics	***Klebsiella pneumoniae* (n=42)**	***Pseudomonas aeruginosa* (n=12)**	***E.coli* (n=10)**	***Acinetobacter baumanii* (n=2)**	***Serratia marscesens* (n=2)**
Amoxycillin	5(11.90%)	NT	2 (20%)	0	0
Amoxycillin - clavulinic acid	25(59.52%)	6 (50%)	5 (50%)	1(50%)	2(100%)
Cotrimoxazole	5 (11.9%)	NT	3(30%)	0	0
Cefoxitin	41(97.61%)	12 (100%)	10(100%)	2(100%)	2(100%)
Cephalexin	11(26.19%)	NT	3(30%)	0	0
Cefotaxime	28(66.66%)	NT	10(100%)	2 (100%)	2(100%)
Ceftazidime	28(66.66%)	11 (91%)	10(100%)	2 (100%)	2(100%)
Ceftriaxone	29(69.04%)	11(91%)	10(100%)	2(100%)	2(100%)
Gentamicin	18(42.85%)	6 (50%)	5(50%)	1(50%)	1(50%)
Amikacin	26(61.90%)	9(75%)	8(80%)	2(100%)	2(100%)
Ciprofloxacin	20(47.61%)	7 (58%)	5(50%)	1(50%)	1(50%)
Ofloxacin	22(52.38%)	9 (75%)	6(60%)	2(100%)	2(100%)
Piperacillin – Tazobactam	41(97.61%)	12 (100%)	10 (100%)	2(100%)	2(100%)
Imipenem	41(97.61%)	11 (91%)	10 (100%)	2(100%)	2(100%)
Meropenem	42(100%)	11(91%)	10 (100%)	2(100%)	2(100%)

As per Table no.12-A, *Klebsiella pneumonia* showed 100% sensitive to Meropenem, 97.61% sensitive to Piperacillin –Tazobactam and Imipenem , 66.66 % sensitive to 3rd generation Cephalosporins (Cefotaxime , Ceftazidime) and Amikacin(61.90%) with least sensitive for Cotrimoxazole (11.90%) and Amoxycillin(11.90%).

TABLE NO: 12 –B: ANTIBIOTIC SENSITIVITY OF GRAM NEGATIVE ISOLATES FROM SPUTUM SAMPLES OF HAP.

Antibiotics	***Klebsiella pneumoniae* (n=12)**	***E.coli* (n=5)**	***Pseudomonas aeruginosa* (n=3)**	***Acinetobacter baumanii* (n=2)**	***Serratia marscesens* (n=1)**	***Proteus mirabilis* (n=1)**
Amoxycillin	1(8.3%)	0	NT	0	0	0
Amoxycillin - clavulinic acid	4(33.3%)	2 (40%)	2 (66.6%)	1(50%)	1(100%)	1(100%)
Cotrimoxazole	2 (16.6%)	1 (20%)	NT	0	0	0
Cefoxitin	10(83.3%)	4 (80%)	NT	NT	1(100%)	1(100%)
Cephalexin	0	0	NT	0	0	0
Cefotaxime	6 (50%)	5(100%)	NT	NT	1(100%)	1(100%)
Ceftazidime	6 (50%)	5(100%)	2 (66.6%)	2 (100%)	1(100%)	1(100%)
Ceftriaxone	6 (50%)	5 (100%)	3 (100%)	2(100%)	1(100%)	1(100%)
Gentamicin	5 (41.6%)	2 (40%)	1 (33.3%)	1(50%)	1(100%)	0
Amikacin	6 (50%)	3 (60%)	2 (66.6%)	2(100%)	1(100%)	1(100%)
Ciprofloxacin	4 (33.3%)	1 (20%)	1 (33.3%)	1(50%)	0	0
Ofloxacin	6(50%)	3 (60%)	2 (66.6%)	2 (100%)	1(100%)	1(100%)
Piperacillin – Tazobactam	11 (91%)	5 (100%)	3 (100%)	2(100%)	1(100%)	1(100%)
Imipenem	12 (100%)	5(100%)	2 (66.6%)	1(50%)	1(100%)	1(100%)
Meropenem	12 (100%)	5 (100%)	3 (100%)	2 (100%)	1(100%)	1(100%)

As per Table no.12-B, *Klebsiella pneumonia*e showed 100% sensitive to Imipenem, Meropenem, 91% sensitive to Piperacillin/Tazobactam, 83.30% sensitive to cefoxitin, 50% sensitive to 3rd generation Cephalosporins (Cefotaxime , Ceftazidime) and Amikacin with least sensitive for Cotrimoxazole (16.66%), Amoxycillin(8.3%) and resistant to cephalexin .

TABLE NO: 12 –C: ANTIBIOTIC SENSITIVITY OF GRAM NEGATIVE ISOLATES FROM ETA SAMPLES OF VAP

Antibiotics	***Klebsiella pneumoniae* (n=6)**	***Acinetobacter baumanii* (n=5)**	***Pseudomonas aeruginosa* (n=5)**	***E.coli* (n=4)**
Amoxycillin	1 (16.6%)	NT	NT	0
Amoxycillin - clavulinic acid	2 (33.3%)	0	1 (20%)	1 (25%)
Cotrimoxazole	0	NT	NT	0
Cefoxitin	5 (83.3%)	NT	NT	3 (75%)
Cephalexin	0	NT	NT	0
Cefotaxime	3 (50%)	NT	NT	3 (75%)
Ceftazidime	3 (50%)	5 (100%)	3 (60%)	3 (75%)
Ceftriaxone	3 (50%)	5 (100%)	3 (60%)	3 (75%)
Gentamicin	1 (16.6%)	2 (40%)	2 (40%)	1 (25%)
Amikacin	3 (50%)	3 (60%)	3 (60%)	2 (50%)
Ciprofloxacin	2 (33.3%)	1 (20%)	1 (20%)	1 (25%)
Ofloxacin	3 (50%)	2 (40%)	3 (60%)	2 (50%)
Piperacillin – Tazobactam	5 (83.3%)	4 (80%)	4 (80%)	4 (100%)
Imipenem	5 (83.3%)	4 (80%)	3 (60%)	3 (75%)
Meropenem	6 (100%)	5 (100%)	3 (60%)	4 (100%)

As per Table no.12-C, *Klebsiella pneumonia*e showed 100% sensitive to Meropenem , 83.30% sensitive to Piperacillin –Tazobactam, Imipenem, Cefoxitin, 50% sensitive to 3rd generation Cephalosporins (Cefotaxime, Ceftazidime), Ofloxacin, Amikacin, with least sensitive to Gentamicin(16.66%), Amoxycillin (16.66%) and resistant to Cotrimoxazole, Cephalexin.

Pseudomonas aeruginosa showed 80% sensitive to Piperacillin Tazobactam, 60% sensitive to Imipenem, Meropenem, 3rd generation Cephalosporins (Ceftazidime, Ceftriaxone), Ofloxacin, Amikacin, with least sensitive to ciprofloxacin (20%).

Acinetobacter baumanii showed 100% sensitive to Meropenem, 3rd generation Cephalosporins (Ceftazidime, Ceftriaxone), 80% sensitive to Piperacillin –Tazobactam, Imipenem, with least sensitive ciprofloxacin (20%).

TABLE NO: 13 – ANTIBIOTIC SENSITIVITY OF GRAM POSITIVE ISOLATES FROM RESPIRATORY SAMPLES OF PNEUMONIA PATIENTS.

Antibiotics	***Staphylococcus aureus***			***Coagulase Negative Staphylococcus aureus***		***Streptococcus pneumoniae***	***Enteroccus faecalis***
	CAP(n=19)	HAP(n=4)	VAP(n=6)	CAP(n=6)	HAP(n=2)	CAP(n=2)	HAP(n=2)
Penicillin G	NT	NT	NT	NT	NT	2(100%)	NT
Amoxycillin	2 (10.5%)	1 (25%)	1 (16.66%)	1 (16.6%)	0	0	0
Erythromycin	12 (63.15%)	2 (50%)	3(50%)	4 (66.6%)	1 (50%)	2 (100%)	1 (50%)
Doxycycline	13 (68.4%)	3 (75%)	3(50%)	4 (66.6%)	1 (50%)	2 (100%)	2 (100%)
Co-trimaxazole	5 (26.3%)	2 (50%)	1(16.66%)	2 (33.3%)	1 (50%	2 (100%)	0
Cefoxitin	16 (84.2%)	3 (75%)	4 (66.66%)	6 (100%)	2 (100%)	0	0
Cephalexin	0	0	0	0	0	0	NT
Cefotaxime	16 (84.20%)	3(75%)	4 (66.66%)	6 (100%)	2 (100%)	2 (100%)	NT
Gentamicin	11 (57.8%)	1 (25%)	2(33.33%)	3 (50%)	1 (50%)	NT	2(HLG) (100%)
Amikacin	15 (78.9%)	3 (75%)	4(66.66%)	4 (66.6%)	1 (50%)	2 (100%)	2 (100%)
Ciprofloxacin	12(63.15%)	2 (50%)	3(50%)	4 (66.6%)	1 (50%)	1 (50%)	1 (50%)
Ofloxacin	16 (84.20%)	3 (75%)	4(66.66%)	5 (83.3%)	1 (50%)	2 (100%)	1 (50%)
Vancomycin	NT	NT	NT	NT	NT	NT	2 (100%)

As per Table no.13, In CAP, *Staphylococcus aureus* showed 84.20% sensitive to Cefoxitin, Cefotaxime and Ofloxacin, least sensitive to Cotrimoxazole(26.30%), Amoxycillin(10.50%) and resistant to Cephalexin.

In HAP, *Staphylococcus aureus* showed 75% sensitive to Cefoxitin, Cefotaxime and Ofloxacin, Amikacin, Doxycycline, least sensitive to Amoxycillin(25%) and resistant to Cephalexin, .

In VAP, *Staphylococcus aureus* showed 66.66% sensitive to Cefoxitin, Cefotaxime and Ofloxacin, Amikacin, least sensitive to Cotrimoxazole (16.66%), Amoxycillin (16.66%) and resistant to Cephalexin.

Streptococcus pneumoniae isolated in CAP showed 100% sensitive to Penicillin G, Cefotaxime, Doxycycline and resistant to Cephalexin, Amoxycillin.

Enterococcus faecalis isolated in HAP showed 100% sensitive to Vancomycin, High level gentamicin, Doxycycline, Amikacin and resistant to Amoxycillin and Cotrimoxazole.

TABLE NO: 14 -A– ANTIBIOTIC SENSITIVITY OF GRAM NEGATIVE ISOLATES FROM BLOOD SAMPLES OF PNEUMONIA PATIENTS.

Antibiotics	***Klebsiella pneumoniae***			***Pseudomonas aeruginosa***	
	CAP	**HAP**	**VAP**	**CAP**	**VAP**
	(n=4)	**(n=1)**	**(n=3)**	**(n=1)**	**(n=1)**
Amoxycillin	1 (25%)	0	0	NT	NT
Amoxycillin - clavulinic acid	3 (75%)	1(100%)	1(33.33%)	0	0
Cotrimoxazole	1 (25%)	0	0	NT	NT
Cefoxitin	4 (100%)	1(100%)	3(100%)	NT	NT
Cephalexin	0	0	0	NT	NT
Cefotaxime	3 (75%)	1(100%)	2(66.66%)	NT	NT
Ceftazidime	4 (100%)	1(100%)	3(100%)	1(100%)	1(100%)
Ceftriaxone	4 (100%)	1(100%)	3(100%)	1(100%)	1(100%)
Gentamicin	1 (25%)	0	1(33.33%)	0	0
Amikacin	2 (50%)	1(100%)	2(66.66%)	0	1(100%)
Ciprofloxacin	1 (25%)	0	1(33.33%)	0	0
Ofloxacin	2 (50%)	1(100%)	2(66.66%)	1(100%)	1(100%)
Piperacillin – Tazobactam	3 (75%)	1(100%)	2(66.66%)	1(100%)	1(100%)
Imipenem	3 (75%)	1(100%)	2(66.66%)	1(100%)	1(100%)
Meropenem	4(100%)	1(100%)	3(100%)	1(100%)	1(100%)

As per Table no.14-A, In CAP *Klebsiella pneumonia*e showed 100% sensitive to Meropenem ,Ceftazidime, Ceftriaxone, Cefoxitin, 25% sensitive to Amoxycillin , Cotrimoxazole, Gentamicin, Ciprofloxacin and resistant to Cephalexin .

In HAP, *Klebsiella pneumonia*e showed 100% sensitive to Piperacillin – Tazobactam, Imipenem, Meropenem**,** 3rd generation Cephalosporins (Cefotaxime,

Ceftazidime), Ofloxacin, Amikacin, cefoxitin and resistant to Amoxycillin, Cotrimoxazole, Gentamicin, Ciprofloxacin, Cephalexin.

In VAP, *Klebsiella pneumonia*e showed 100% sensitive to Meropenem, Ceftazidime, Ceftriaxone, Cefoxitin and resistant to Amoxycillin and Cotrimoxazole, Cephalexin.

Pseudomonas aeruginosa isolated in CAP and VAP showed 100% sensitive to Piperacillin –Tazobactam, Imipenem, Meropenem**,** Ceftazidime, Ceftriaxone , Ofloxacin, Amikacin and resistant to Gentamicin, Ciprofloxacin.

TABLE NO: 14 -B– ANTIBIOTIC SENSITIVITY OF GRAM POSITIVE ISOLATES FROM BLOOD SAMPLES OF PNEUMONIA PATIENTS.

Antibiotics	***Staphylococcus aureus***			***Coagulase Negative Staphylococcus aureus***
	CAP(n=2)	HAP(n=1)	VAP(n=1)	CAP(n=1)
Amoxycillin	0	0	0	0
Erythromycin	1 (50%)	0	0	0
Doxycycline	2 (100%)	1 (100%)	0	1 (100%)
Co-trimaxazole	1 (50%)	0	0	0
Cefoxitin	2 (100%)	1 (100%)	1 (100%)	1 (100%)
Cephalexin	0	0	0	0
Cefotaxime	2 (100%)	1 (100%)	1 (100%)	1 (100%)
Gentamicin	1 (50%)	0	0	0
Amikacin	1 (50%)	1 (100%)	1 (100%)	1 (100%)
Ciprofloxacin	1 (50%)	0	0	0
Ofloxacin	2 (100%)	1 (100%)	1 (100%)	1 (100%)

As per Table no.14-B, In CAP, *Staphylococcus aureus* showed 100% sensitive to Cefoxitin, Cefotaxime and Ofloxacin, Doxycycline and resistant to Cephalexin, Amoxycillin.

In HAP, *Staphylococcus aureus* showed 100% sensitive to Cefoxitin, Cefotaxime, Ofloxacin, Amikacin, Doxycycline, and resistant to Cephalexin, Amoxycillin, Cotrimoxazole, Erythromycin, Gentamicin, Ciprofloxacin.

In VAP, *Staphylococcus aureus* showed 100% sensitive to Cefoxitin, Cefotaxime and Ofloxacin, Amikacin, and resistant to Cephalexin, Amoxycillin and Cotrimoxazole and resistant to Cephalexin, Amoxycillin, Cotrimoxazole, Gentamicin, Ciprofloxacin.

TABLE NO: 15– CHARACTERISATION OF ESBL PRODUCING *KLEBSIELLA PNEUMONIAE*

PNEUMONIA	PHENOTYPIC METHOD		GENOTYPIC METHOD		
	SCREENING POSITIVE	PHENOTYPIC CONFIRMATORY	CTX-M	TEM	SHV
CAP(n=42)	13(30.95%)	11(26.19%)	11	9	10
HAP (n=12)	6(50%)	4(33.33%)	4	2	4
VAP (n=6)	3(50%)	3(50%)	3	2	3
TOTAL	22	18	18	13	17

As per Table no.15, Distribution of ESBL producing *Klebsiella pneumoniae* in CAP 11 (26.19%) , HAP 4 (33.33%) and VAP 3(50%) which is confirmed by phenotypic and genotypic methods.

TABLE NO 16–MINIMUM INHIBITORY CONCENTRATION OF ISOLATES OF ESBL KLEBSIELLA PNEUMONIAE TO CEFTAZIDIME AND CEFTAZIDIME CLAVULINIC ACID (µg/ml) (n=18)

Phenotypic Confirmation of ESBL by E – Strip test

Name of the Organism	No. Of Strains	Ceftazidime MIC	Ceftazidime + Clavulanic Acid MIC	Result
K.pneumoniae	12	>32	0.25	Positive
	6	6	0.125	Positive

As per table no: 16, 18 isolates of ESBL producing *Klebsiella pneumoniae* tested for MIC by E-test. MIC value for 12 isolates is >32µg/ml for ceftazidime and 0.25 µg/ml for ceftazidime-clavulanic acid. MIC value for 6 isolates is 6 µg /ml for ceftazidime and 0.125 µg/ml for ceftazidime-clavulanic acid. As per CLSI guidelines 2015, MIC value of ceftazidime – clavulanate should be ≥ 8 fold decrease in concentration than MIC value of ceftazidime alone.

TABLE NO: 17-DETECTION OF MBL PRODUCING *PSEUDOMONAS AERUGINOSA* IN PNEUMONIA

PNUEMONIA	MBL DETECTION
CAP(n=12)	1(8.3%)
HAP(n=3)	0
VAP(n=5)	2 (40%)

As per Table no.17, Detection of MBL Producing *Pseudomonas aeruginosa* in 1(8.3%) CAP and in VAP 2 (40%).

TABLE NO: 18 – CHARACTERISATION OF Amp-C PRODUCING ENTEROBACTERIACEA IN PNEUMONIA

PNEUMONIA	*KLEBSIELLA PNEUMONIAE*			*ESCHERICHIA COLI*		
	No.	**SCREENING POSITIVE**	**CONFIRMATORY**	**No.**	**SCREENING POSITIVE**	**CONFIRMATORY**
CAP	42	1 (2.3%)	1 (2.3%)	10	0	0
HAP	12	2(16.66%)	2(16.66%)	5	1(20%)	1(20%)
VAP	6	1(16.66%)	1(16.66%)	4	1(25%)	1(25%)

As per Table no.18, Distribution of Amp-C producing Enterobacteriacea in pneumonia showing resistance to Cefoxitin 30µg (<18mm) as follows: CAP showed 1 (2.3%) were detected as Amp-C producing *Klebsiella pneumoniae*. HAP showed 2 (16.66%) were detected as Amp-C producing *Klebsiella pneumoniae* and 1 (20%) were detected as Amp-C producing *Escherichia coli.* VAP showed 1 (16.66%) were detected as Amp-C producing *Klebsiella pneumoniae* and 1 (25%) were detected as Amp-C producing *Escherichia coli.*

TABLE NO: 19 – CHARACTERISATION OF MRSA

PNEUMONIA	PHENOTYPIC METHOD				GENOTYPIC METHOD
	SCREENING POSITIVE	Vancomycin MIC E-TEST			mec-A
		≤2µg/ml	4-8 µg/ml	≥16µg/ml	
CAP(n=19)	3 (15.78%)	3	-	-	3 (15.78%)
HAP(n=4)	1 (25%)	1	-	-	1 (25%)
VAP(n=6)	2(33.33%)	2	-	-	2(33.33%)
TOTAL	6	6	-	-	6

As per Table no.19, Distribution of MRSA in CAP 3 (15.78%), HAP 1(25%) and 2 (33.33%) which is confirmed by Phenotypic method - Cefoxitin disc diffusion test (cefoxitin zone size ≤ 21mm) and mec A gene was positive for all the isolates of MRSA by genotype methods. All isolates of MRSA showed MIC value to Vancomycin <2µg/ml, so it rules out Vancomycin Resistant *Staphylococcus aureus* (VRSA).

TABLE NO -20: OUTCOME OF THE STUDY (n=205)

OUTCOME	CAP(n=150)	HAP(n=30)	VAP(n=25)
RECOVERED	95(63.33%)	18(60%)	13(52%)
EXPIRED	15(10%)	7(23.33%)	12(48%)
LOST FOLLOW UP	40(26.66%)	5(16.66%)	0

As per Table no.20, Outcome of the study in pneumonia patients were among 40(26.66%) lost follow up, 95 (63.33%) recovered, 15(10%) expired in CAP. In HAP 5(16.66%) lost follow up, 18(60%) recovered and 7(23.33%) expired. In VAP 13(52%) recovered, 12(48%) expired.

CHART 6
DISTRIBUTION OF ESBL PRODUCING *KLEBSIELLA PNEUMONIAE* IN PNEUMONIA

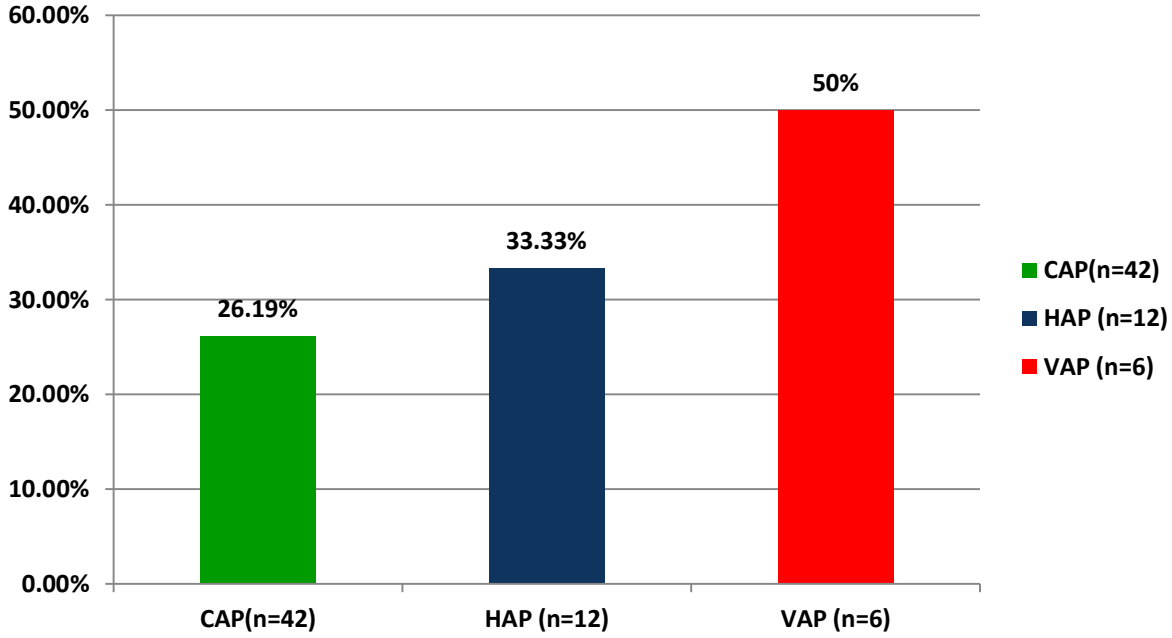
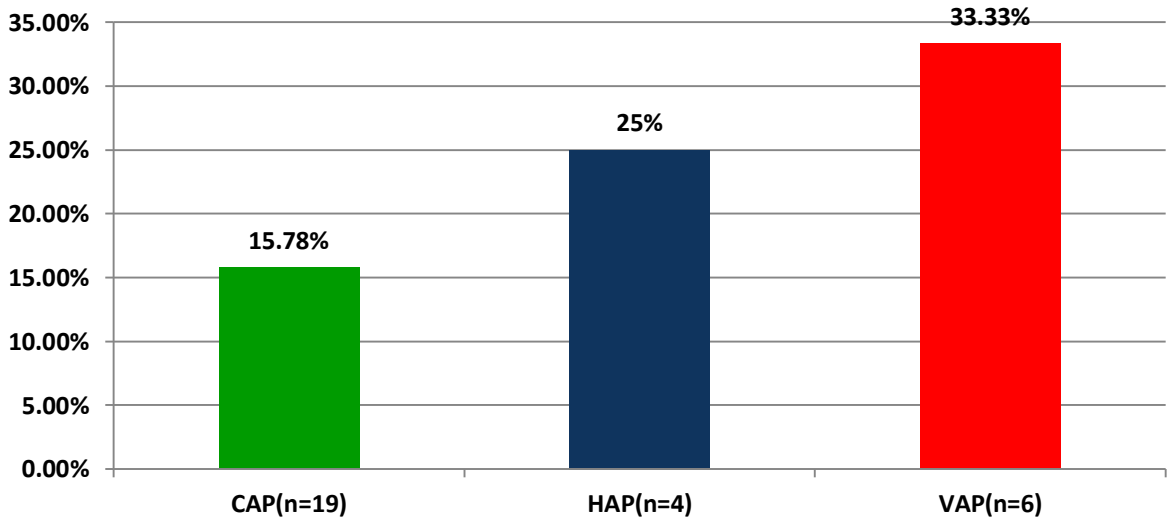
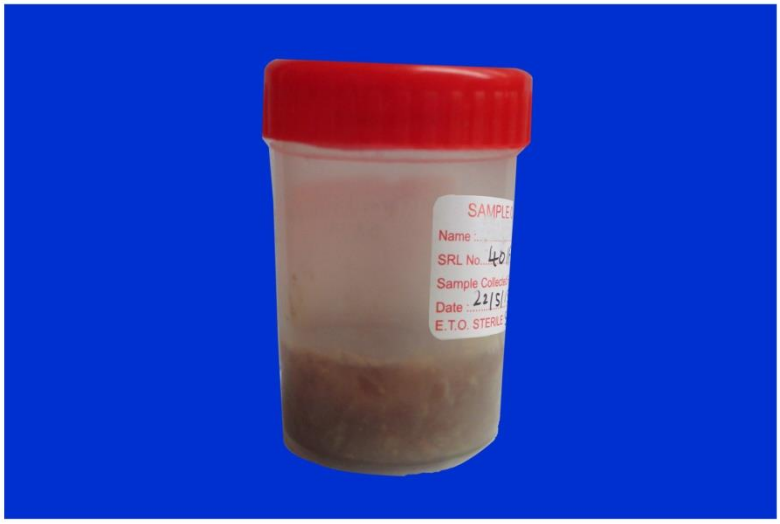


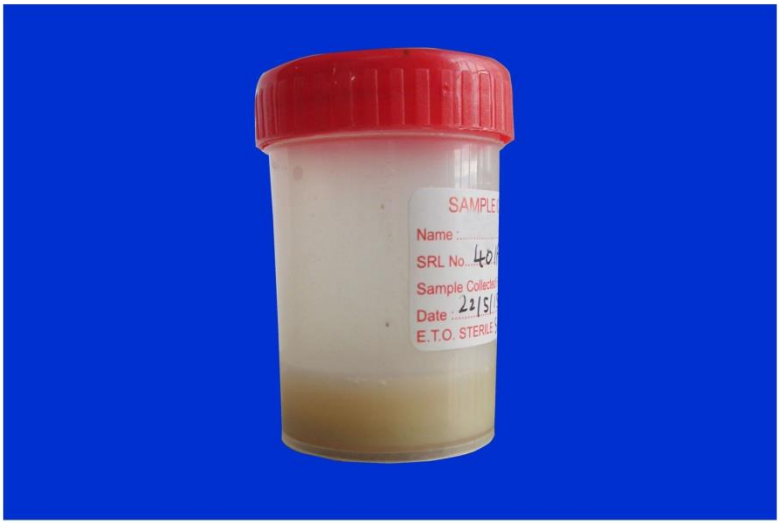
CHART 7
DISTRIBUTION OF MRSA IN PNEUMONIA



SPUTUM COLLECTION

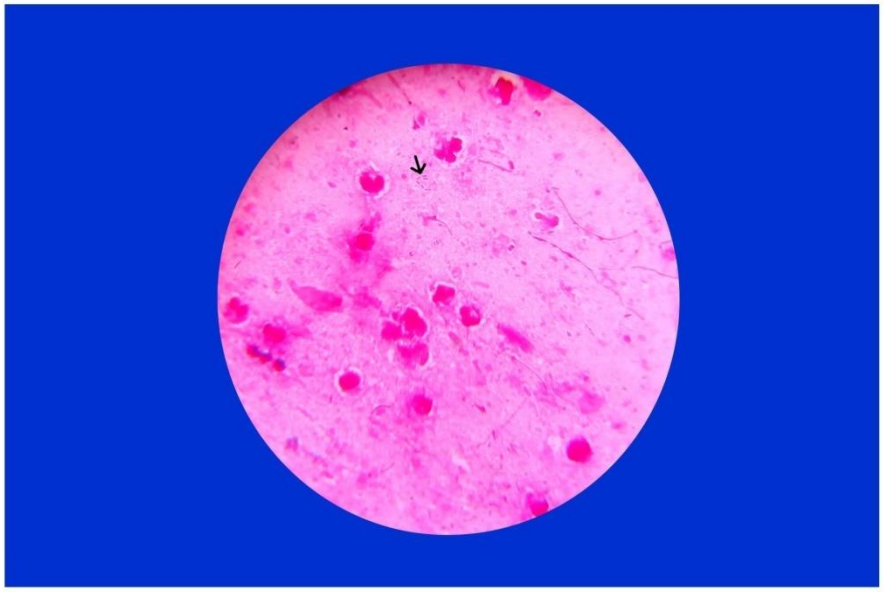


A - RED CURRANT JELLY SPUTUM

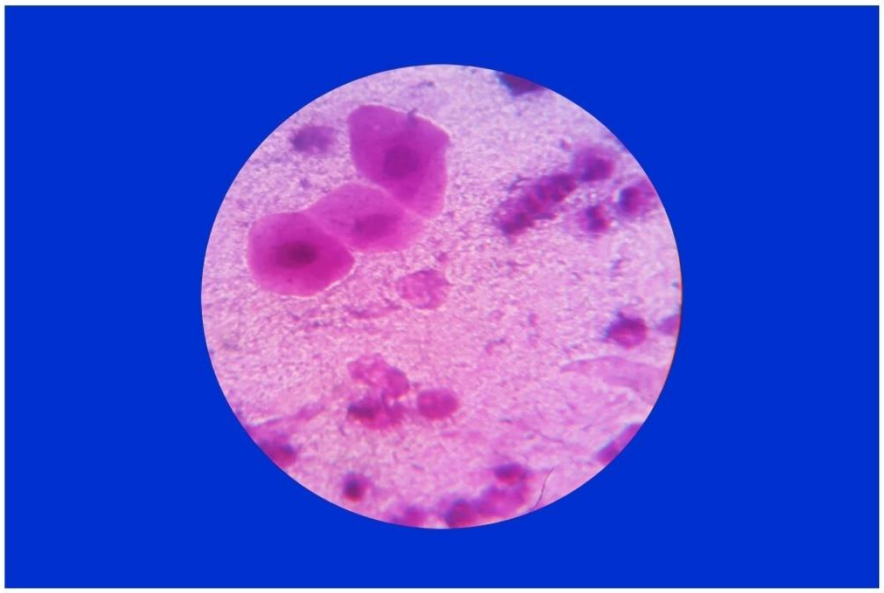


B - MUCOPURULENT SPUTUM

DIRECT GRAM STAIN - SPUTUM



A - PLENTY OF PUS CELLS, GNB SEEN

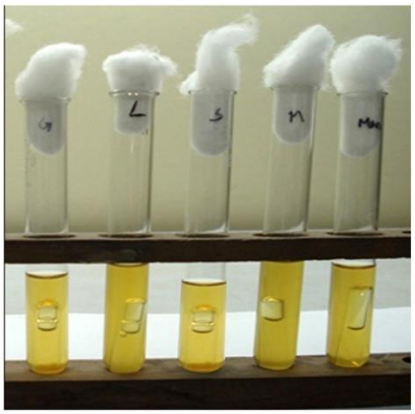


B - PLENTY OF PUS CELLS, 3 SQUAMOUS EPITHELIAL CELLS SEEN

KLEBSIELLA PNEUMONIAE - MAC CONKEY AGAR

LACTOSE FERMENTING MUCOID COLONIES

BIOCHEMICAL REACTIONS OF KLEBSIELLA PNEUMONIAE

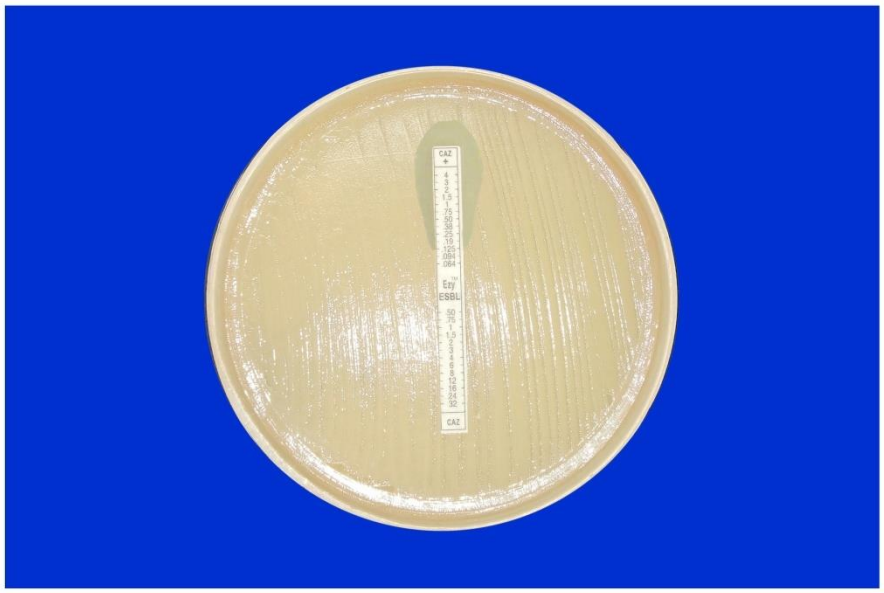


PHENOTYPIC CONFIRMATORY TEST FOR ESBL DETECTION

CAZ = Ceftazidime 30 µg

CAZ + CLAV = Ceftazidime 30µg +clavulanic acid 10 µg(≥ 5mm than CAZ)

ESBL DETECTION BY E-STRIP METHOD



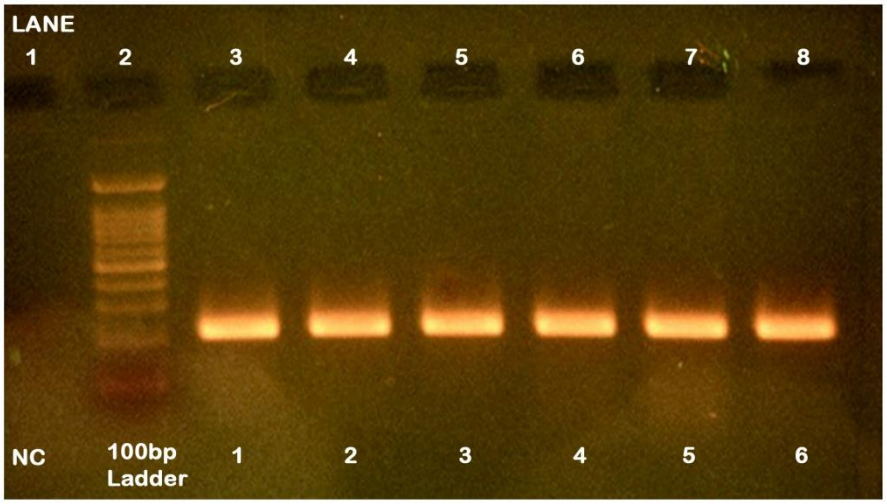
MIC VALUE OF CEFTAZIDIME - CLAVULANATE SHOWS 8 FOLD DECREASED CONCENTRATION THAN CEFTAZIDIME ALONE

AMPC DISK TEST

FLATTENING OF ZONE SIZE TOWARDS AMPC PRODUCING *K.pneumoniae*

DETECTION OF ESBL GENE BY PCR

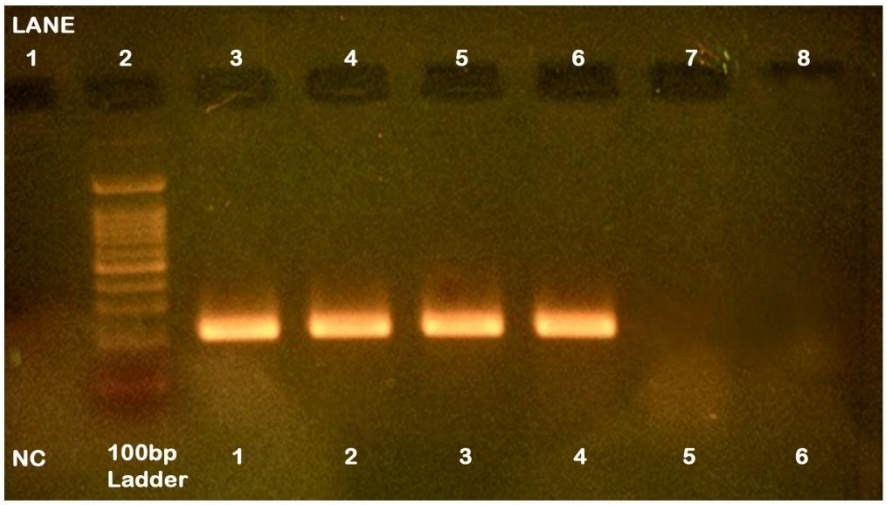
CTX-M



LANE 1	**NEGATIVE CONTROL**
LANE 2	**100bp LADDER**
LANE 3-8	**SAMPLES WITH CTX-M PRIMER**
LANE 3-8	**POSTIVE FOR CTX-M GENE**

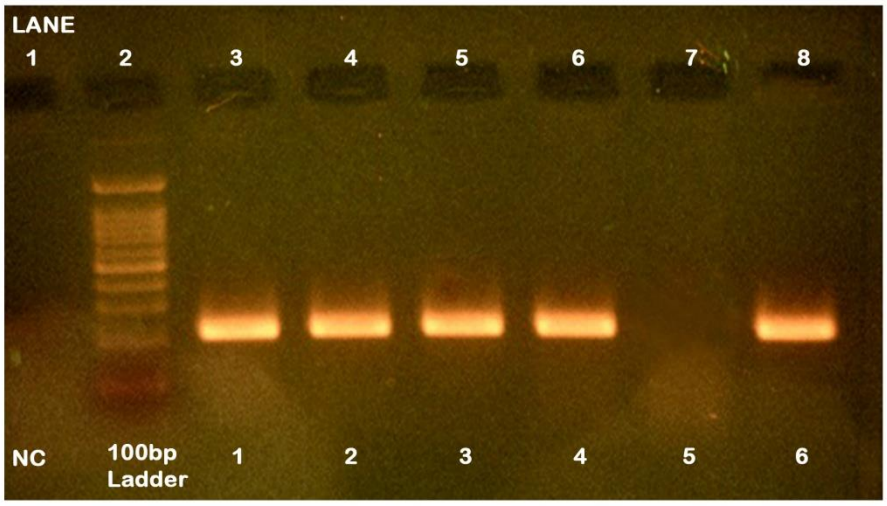
DETECTION OF ESBL GENE BY PCR

TEM GENE



LANE 1	NEGATIVE CONTROL
LANE 2	100bp LADDER
LANE 3-8	SAMPLES WITH TEM PRIMER
LANE 3-6	POSTIVE FOR TEM GENE

SHV GENE

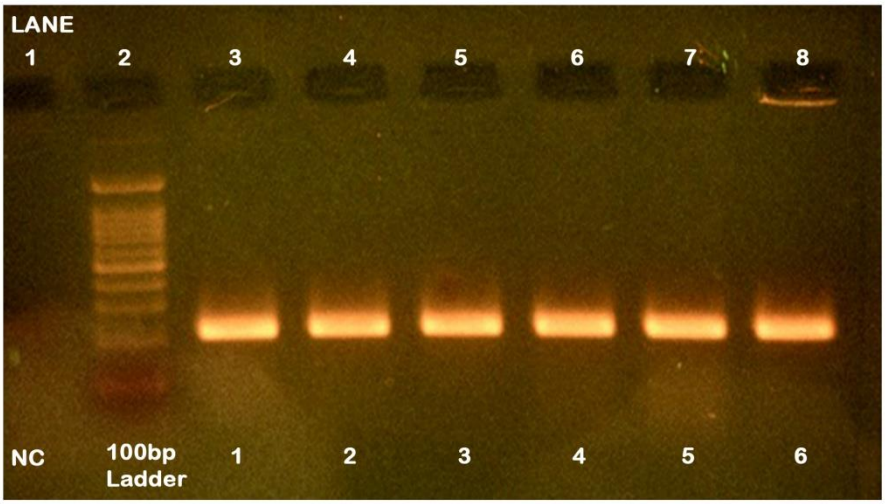


LANE 1	NEGATIVE CONTROL
LANE 2	100bp LADDER
LANE 3-8	SAMPLES WITH SHV PRIMER
LANE 3,4,5,6,8	POSTIVE FOR SHV GENE

VANCOMYCIN MIC OF MRSA - E-TEST

MIC VALUE IS 1.5 (<2) INDICATES VANCOMYCIN SENSITIVE *S. aureus*

DETECTION OF mec A GENE BY PCR



LANE 1	NEGATIVE CONTROL
LANE 2	100bp LADDER
LANE 3-8	SAMPLES WITH mec A PRIMER
LANE 3-8	POSTIVE FOR mec A GENE

DISCUSSION

Pneumonia remains a significant medical problem in both community as well as in hospital. Despite the advent of antibiotics, improved diagnostic, microbial techniques and sophisticated respiratory support system effective treatment of pneumonia still poses a huge challenge**.** This study aims at detecting the common bacterial isolates causing Pneumonia in adults with their antibiotic susceptibility pattern, Methicillin Resistant *Staphylococcus aureus* (MRSA) and Extended spectrum beta lactamase (ESBL) producers in patients attending Government Kilpauk Medical College and Hospital with clinical symptoms and radiological evidence of pneumonia. The observations were recorded, analysed and discussed as follows.

As per Table no.1, Patients with age group of 40- 60 years were highly affected by Community acquired pneumonia (CAP) and Ventilator associated pneumonia (VAP). CAP was 70 (46.66%) which is similar to study done by Vishak K Acharya et al [3](41%). VAP was 14(56%) which is in contrast to study of Saroj Golia et al[7]18 (39.13%). Whereas in Hospital acquired pneumonia (HAP) more number of cases was detected in age group above 60 years 13(43.33%).

As per Table no.2, Adult males were commonly affected by pneumonia , which in CAP was 68% which is similar to study done by Sandeep Kumar Jain et al[28] (67.50%) and in contrast to Vishak K Acharya et al [3] (38%) .

In HAP, 20 (66.66%) of males were affected which is similar to the study of Noyal Mariya Joseph et al [37] (57.90%).

In VAP, 16 (64%) of males were affected which coincides with the study of Neelima Ranjan Uma et al[91] 65%, Masoumeh bagheri- Neasmi et al[9] 68.20%, Kotgire Santhosh A et al.[13] 61.53%. This is because smoking being the most common risk factor, men are commonly affected.

As per Table no.3, Smoking is the common risk factor in CAP 48.66% which coincides with Sandeep Kumar Jain et al[28] 40.80% and is in contrast to the study of A.V,Sowmya et al[4] in which diabetes is the common risk factor with 48% . COPD and diabetes were the common risk factor in study by Vishak K Acharya et al [3] 10.50%. This is because tobacco smoking may lead to impairment of mucociliary function thereby allowing the pathogens to survive[85]. Type 2 Diabetes mellitus is also one of the important risk factor in CAP followed by COPD and alcoholism.

As per Table no.4, Cough is the most common presenting symptom in 94.66% of CAP which coincides with Sandeep Kumar Jain et al[28] 92.50%, other symptoms being fever, breathlessness and chestpain.

In HAP 86.66% had cough as the presenting symptom.

VAP is based on clinical criteria (Modified CPIS- Modified Clinical Pulmonary Infection Score >6)[8].

As per Table no. 5, Right lower lobe was the commonly involved lung lobe seen in Chest radiography of pneumonia patients with 36% in CAP coinciding with Sandeep Kumar Jain et al[28] 48.30%.

In HAP 33.33% had Right lower lobe involvement.

In VAP 36% showed Right lower lobe involvement.

As per Table no.6: Respiratory samples (Sputum & ETA) collected from pneumonia patients showed growth in culture as follows:

In CAP 83(55.33%) showed growth in sputum culture which was similar to the study of Sushma Sawaraj et al[92] 62% and in contrast to Vishak K Acharya et al[3] 39%, Sandeep Kumar Jain et al[28] 36.70%.

In HAP 26(86.66%) showed growth in sputum culture.

In VAP 25(100%) showed growth in Endotracheal aspirate culture coinciding with study of Saroj Golia et al[7]52 (100%).

Bacteremia being one of the complications of Pneumonia, it's important to do blood culture in pneumonia patients with fever (temp>37.8ºC). In CAP 8(10%) showed growth in Blood culture which coincides with Sandeep Kumar Jain et al[28] 9.2%, A.V. Sowmya et al[4] 9.33%.

As per Table no.7- Direct gram stain of respiratory samples is used to determine whether a sample is representative of the site of infection or contamination as per Barlett's grading[48,50]. Respiratory samples with direct smear positive showing culture positive were 80(53.33%) of CAP, 24(80%) of HAP and 21(84%) of VAP.This is similar to the study done by Mariaraj et al[93] (63.20%), Anuradha Mokkapati et al[94] (89.74%). 3(2%) samples of CAP, 2(6.66%) of HAP and 4(16%) of VAP showed direct smear negative but culture positive indicating that culture is

the gold standard. So Gram staining can be used as preliminary screening technique.

As per Table no.8, Culture growth from respiratory samples of pneumonia patients showed results as follows:

- In CAP, 71(85.54%) showed monomicrobial growth and 12(14.45%) showed Polymicrobial growth.
- In HAP, 20(76.92%) showed monomicrobial growth in culture and 6(23.07%) showed polymicrobial growth which was in contrast to Vasuki et al[18] were 8.3% showed polymicrobial growth.
- In VAP, 24(96%) showed monomicrobial growth and 1(4%) showed polymicrobial growth . Similar observation was seen in the study of S Qureshi et al [8] were 94.74% was monomicrobial and 5.26% was polymicrobial. This is in contrast to study of Saroj Golia et al[7] were 86.54% was monomicrobial ,13.46% polymicrobial and Kotgire Santhosh A et al.[13] were 43% was monomicrobial and 56% polymicrobial .

As per Table no. 9, Among the organisms isolated from respiratory samples of pneumonia patients , Gram negative bacilli was the predominant isolate in 68(71.57%) samples of CAP, 24(75%) of HAP. In VAP 20(76.92%) were Gram negative bacilli which is similar to the study of Neelima Ranjan Uma et al[91] 95.70%. Gram positive cocci accounts for about 27(28.42%) in CAP, 8(25%) in HAP, 6 (23.07%) in VAP.

Gram negative bacilli was also the predominant isolate in blood culture of CAP (62.50%) and VAP (80%). Gram positive cocci was about 3(37.50%) in CAP, 1(50%) in HAP and 1(20%) in VAP.

As per Table no.10, *Klebsiella pneumoniae* was the predominant organism grown in respiratory samples:

- In CAP, 42 (44.21%) were isolated which coincides with the study of A.V,Sowmya et al[4] 34.62% and was in contrast to Sandeep Kumar Jain et al[28,] and Vishak K Acharya et al [3] where *Streptococcus pneumoniae* was the predominant organism followed by *Klebsiella pneumoniae* .
- In HAP, 12 (37.50%) were *Klebsiella pneumonia,* which coincides with study of Vasuki et al[18] 48.20%.
- In VAP, both *Klebsiella pneumoniae and Staphylococcus aureus* were isolated in 6(23.07%) samples which coincides with the study of Kotgire Santhosh A et al.[13] with 16.21% and 20.27% respectively .

Among Gram positive cocci, *Staphylococcus aureus* isolated in 19(20%) samples of CAP, 4(12.50%) of HAP and 6(23.07%) of VAP.

Among non-fermenters, *Pseudomonas aeruginosa* was isolated in 12(12.63%) samples of CAP, 3(9.37%) of HAP and 5(19.23%) of VAP.

As per Table no.11, *Klebsiella pneumoniae* was the predominant organism isolated in blood culture of 4 (50%) samples of CAP which was similar to the study of A.V.Sowmya et al[4] 50% and also from 3(60%) samples of VAP.

As per Table no.12-A, Antibiotic sensitivity pattern of Gram negative isolates in CAP were as follows:

- *Klebsiella pneumoniae* showed 100% sensitive to Meropenem , 97.61% sensitive to Piperacillin –Tazobactam and Imipenem , 66.66 % sensitive to 3rd generation Cephalosporins (Cefotaxime , Ceftazidime) and Amikacin(61.90%) with least sensitive for Cotrimoxazole (11.90%) and Amoxycillin(11.90%). while study done by Sushma Sawaraj et al[92] showed 100% sensitivity to Imipenem.
- *Pseudomonas aeruginosa* was 100% sensitive to Piperacillin –Tazobactam and Cefoxitin, 91% sensitive to Imipenem, Meropenem and 3rd generation Cephalosporins (Ceftazidime, Ceftriaxone) with least sensitive to Gentamicin (50%), while study done by by Sushma Sawaraj et al [92] showed 90% sensitivity to 3rd generation Cephalosporins and 50% sensitivity to Imipenem .
- *Escherichia coli* was 100% sensitive to Piperacillin –Tazobactam, Imipenem, Meropenem, 3rdgeneration Cephalosporins (Cefotaxime, Ceftazidime) and Cefoxitin, 80% sensitive to Amikacin, 60% sensitive to Ofloxacin, 50%sensitive to Amoxyclavulanic acid, Gentamicin and ciprofloxacin , 30% sensitive to Cotrimoxazole and Cephalexin, whereas in study done by by Sushma Sawaraj et al[92] it showed 81.80% sensitivity to Imipenem .

As per Table no.12-B, Antibiotic sensitivity pattern of Gram negative isolates in HAP were as follows:

- *Klebsiella pneumonia*e showed 100% sensitive to Imipenem, Meropenem, 91% sensitive to Piperacillin/Tazobactam, 83.30% sensitive to cefoxitin, 50% sensitive to 3rd generation Cephalosporins (Cefotaxime, Ceftazidime) and Amikacin with least sensitive for Cotrimoxazole(16.66%), Amoxycillin(8.3%) and cephalexin(0). In the study done by Vasuki et al [18], *Klebsiella pneumonia*e showed 100% sensitivity to Imipenem.
- *Escherichia coli* showed 100% sensitivity to Piperacillin –Tazobactam, Imipenem, Meropenem and 3rd generation Cephalosporins (Cefotaxime, Ceftazidime), resistant to Amoxycillin and cephalexin. In the study done by Vasuki et al [18], *Escherichia coli* showed 100% sensitivity to Imipenem .
- *Pseudomonas aeruginosa* was 100% sensitive to Piperacillin –Tazobactam, Meropenem and Ceftriaxone, and was least sensitive to ciprofloxacin (33.30%) and Gentamicin (33.30%). In the study done by Vasuki et al[18], *Pseudomonas aeruginosa* showed 100% sensitivity to Imipenem.

As per Table no.12-C, Antibiotic sensitivity pattern of Gram negative isolates in VAP as follows:

- *Klebsiella pneumonia*e showed 100% sensitive to Meropenem, 83.30% sensitive to Piperacillin –Tazobactam, Imipenem, Cefoxitin, 50% sensitive to 3rd generation Cephalosporins (Cefotaxime, Ceftazidime), Ofloxacin, Amikacin, with least sensitive to Gentamicin(16.66%), Amoxycillin(16.66%), Cotrimoxazole(0) and Cephalexin(0).whereas study done by Saroj Golia et al[7], 100% sensitive to Piperacillin –Tazobactam,

Meropenem, Imipenem. S Qureshi et al[8] 100% sensitive to Meropenem and Imipenem, 77% sensitive to Piperacillin –Tazobactam.

- *Pseudomonas aeruginosa* showed 80% sensitive to Piperacillin Tazobactam, 60% sensitive to Imipenem, Meropenem, 3rd generation Cephalosporins (Ceftazidime, Ceftriaxone), Ofloxacin, Amikacin, with least sensitive to ciprofloxacin(20%) in this study whereas study done by Saroj Golia et al[7], *Pseudomonas aeruginosa* showed 45% sensitive to Piperacillin-Tazobactam, Ceftazidime, 60% sensitive to Meropenem, Imipenem. S Qureshi et al [8] 77% sensitive to Meropenem, Imipenem and Piperacillin-Tazobactam, 44% sensitive to 3rd generation Cephalosporins (Ceftazidime).
- *Acinetobacter baumanii* showed 100% sensitive to Meropenem, 3rd generation Cephalosporins(Ceftazidime, Ceftriaxone), 80% sensitive to Piperacillin –Tazobactam, Imipenem, with least sensitive ciprofloxacin (20%) in this study, whereas study done by Saroj Golia et al[7], *Acinetobacter baumanii* showed 62.50% sensitive to Meropenem, Imipenem, 25% sensitive to Piperacillin –Tazobactam, S Qureshi et al[8] showed 100% sensitive to Piperacillin –Tazobactam, Meropenem, Imipenem.
- *Escherichia coli* showed 100% sensitive to Piperacillin –Tazobactam , Meropenem,75% sensitive to Imipenem, 3rd generation Cephalosporins (Cefotaxime,Ceftazidime), Cefoxitin, 50% sensitive to Amikacin and Ofloxacin, 25% sensitive to Amoxycillin-clavulanic acid, Gentamicin, ciprofloxacin and resistant to cotrimoxazole, cephalexin, Amoxycillin in this study, whereas study done by Saroj Golia et al[7], *Escherichia coli* showed

100% sensitive to Piperacillin –Tazobactam, Meropenem, Imipenem, S Qureshi et al [8] 100% sensitive to Meropenem, Imipenem ,77% sensitive to Piperacillin –Tazobactam.

As per Table no.13, Gram positive organism isolated from respiratory samples of pneumonia patients as follows:

- In CAP, *Staphylococcus aureus* showed 84.20% sensitive to Cefoxitin, Cefotaxime and Ofloxacin, least sensitive to Cotrimoxazole(26.30%), Amoxycillin(10.50%) and resistant to Cephalexin. whereas study done by Sushma Sawaraj et al[92] showed 82.30% sensitive to Cefoxitin.
- In HAP, *Staphylococcus aureus* showed 75% sensitive to Cefoxitin, Cefotaxime and Ofloxacin, Amikacin, Doxycycline, least sensitive to Amoxycillin(25%) and resistant to Cephalexin. whereas study done by Vasuki et al [18], *Staphylococcus aureus* showed 93.54% sensitive to Cefotaxime.
- In VAP, *Staphylococcus aureus* showed 66.66% sensitive to Cefoxitin, Cefotaxime and Ofloxacin, Amikacin, least sensitive to Cotrimoxazole (16.66%) , Amoxycillin (16.66%) and resistant to Cephalexin, whereas study done by Saroj Golia et al[7], *Staphylococcus aureus* showed 28.57% sensitive to Erythromycin, 14.29% sensitive to Cefoxitin. Study done by S Qureshi et al[8] showed 50% sensitive to Cefoxitin, Erythromycin, Doxycycline, Gentamicin and resistant to Cotrimoxazole.
- *Streptococcus pneumoniae* isolated in CAP showed 100% sensitive to Penicillin G, Cefotaxime, Doxycycline and resistant to Cephalexin,

Amoxycillin. whereas study done by Sushma Sawaraj et al[92] showed 56% sensitive to Cefotaxime, 18.7% sensitive to Penicillin G.

- *Enteroccus faecalis* showed 100% sensitive to Vancomycin, High level gentamicin, Doxycycline, Amikacin and resistant to Amoxycillin and Cotrimoxazole.

As per Table no.14-A, Gram negative organism isolated from blood samples of pneumonia patients as follows:

In CAP *Klebsiella pneumonia*e showed 100% sensitive to Meropenem, Ceftazidime, Ceftriaxone, Cefoxitin, 25% sensitive to Amoxycillin, Cotrimoxazole, Gentamicin, Ciprofloxacin and resistant to Cephalexin.

In HAP, *Klebsiella pneumonia*e showed 100% sensitive to Piperacillin – Tazobactam, Imipenem, Meropenem**,** 3rd generation Cephalosporins (Cefotaxime, Ceftazidime), Ofloxacin, Amikacin, cefoxitin and resistant to Amoxycillin, Cotrimoxazole, Gentamicin, Ciprofloxacin, Cephalexin.

In VAP *Klebsiella pneumonia*e showed 100% sensitive to Meropenem, Imipenem, Ceftazidime, Ceftriaxone, Cefoxitin and resistant to Amoxycillin and Cotrimoxazole, Cephalexin.

Pseudomonas aeruginosa isolated in CAP and VAP showed 100% sensitive to Piperacillin –Tazobactam, Imipenem, Meropenem**,** Ceftazidime, Ceftriaxone, Ofloxacin, Amikacin and resistant to Gentamicin, Ciprofloxacin.

As per Table no.14-B, Gram positive organism isolated from blood samples of pneumonia patients as follows:

- In CAP, *Staphylococcus aureus* showed 100% sensitive to Cefoxitin, Cefotaxime and Ofloxacin, Doxycycline and resistant to Cephalexin, Amoxycillin.

- In HAP, *Staphylococcus aureus* showed 100% sensitive to Cefoxitin, Cefotaxime, Ofloxacin, Amikacin, Doxycycline, and resistant to Cephalexin, Amoxycillin, Cotrimoxazole, Erythromycin, Gentamicin, Ciprofloxacin.
- In VAP, *Staphylococcus aureus* showed 100% sensitive to Cefoxitin, Cefotaxime and Ofloxacin, Amikacin, and resistant to Cephalexin, Amoxycillin and Cotrimoxazole and resistant to Cephalexin, Amoxycillin, Cotrimoxazole, Gentamicin, Ciprofloxacin.

As per Table no.15, Extended spectrum betalactamases has lead to ineffectiveness of antibiotics and increase in the severity of illness due to various pathogenic strain causing pneumonia[11]. Screening of ESBL was done by disk diffusion method (30.95% in CAP, 50% in HAP & 50% in VAP) and it is confirmed by phenotypic confirmatory disk diffusion method.

- In CAP, 11(26.19%) was ESBL *K.pneumoniae* with CTX-M is common gene obtained in this study which coincides with the study of Arijit Bora et al[90].
- In HAP, 4(33.33%) which is contrast to the study of Vasuki et al[18] 82%.

- InVAP, 3 (50%) which is contrast to the study of S Qureshi et al [8] 75%, Kotgire Santhosh A et al.[13] 33.33%, Veena Krishna Murthy et al[11] 32.10%.Genotypic study of ESBL *K.pneumoniae* isolates in VAP coincides with the study of Veena Krishna Murthy et al[11].CTX-M is the predominant gene isolated followed by TEM and SHV gene in Genotypic study of ESBL *K.pneumoniae.*

As per table no: 16, 18 isolates of ESBL producing *Klebsiella pneumoniae* tested for MIC by E-test. MIC value for 12 isolates is >32µg/ml for ceftazidime and 0.25 µg/ml for ceftazidime-clavulanic acid. MIC value for 6 isolates is 6 µg /ml for ceftazidime and 0.125 µg/ml for ceftazidime-clavulanic acid. As per CLSI guidelines 2015, MIC value of ceftazidime – clavulanate should be ≥ 8 fold decrease in concentration than MIC value of ceftazidime alone.

As per Table no.17, Meropenem: 10µg (MRP) (Zone of inhibition ≤ 18mm) resistant *Pseudomonas aeruginosa* isolates was taken for screening MBL production. Antibiotic sensitivity testing was done by the Kirby-Bauer disc diffusion method.

MBL Producing *Pseudomonas aeruginosa* was detected by isolates showing resistance to Meropenem 10µg (Zone of inhibition ≤18mm) as follows: 1(8.3%) CAP. 2 (40%) in VAP as MBL Producing *Pseudomonas aeruginosa* which coincide with the study of Saroj Golia et al[7] 40%, S Qureshi et al [8] 37.50% and contrast to the study Kotgire Santhosh A et al.[13] 1.5%

As per Table no.18, Distribution of Amp-C producing Enterobacteriacea in pneumonia showing resistance to Cefoxitin (<18mm) as follows:

- CAP showed 1 (2.3%) were detected as Amp-C producing *Klebsiella pneumonia.*
- HAP showed 2(16.66%) were detected as Amp-C producing *Klebsiella pneumoniae* and 1 (20%) were detected as Amp-C producing *Escherichia coli.*
- VAP showed 1(16.66%) were detected as Amp-C producing *Klebsiella pneumoniae* which is in contrast to the study of Kotgire Santhosh A et al.[13] 33.33%. 1(25%) were detected as Amp-C producing *Escherichia coli* in VAP which coincides with the study of Noyal Mariya Joseph et al[95] 25%.

As per Table no.19, Early detection and treatment of MRSA is very important to prevent complications and fatal outcomes. Distribution of MRSA in Pneumonia patients as follows: 3(15.78%) in CAP which coincides with the study of Sushma Sawaraj et al[92] 17.64%.

1(25%) MRSA in HAP which is in contrast to the study of Vasuki et al[18] 6.20% and Chiranjoy Mukhopadhyay et al [34] 53.30%.

2(33.33%) in VAP were MRSA in this study whereas MRSA in other studies as follows: Kotgire Santhosh A et al.[13] 53.33%, S Qureshi et al [8] 66.70% and Saroj Golia et al[7] 85.71%. All isolates of MRSA showed MIC value to Vancomycin <2µg/ml, so it rules out Vancomycin Resistant *Staphylococcus aureus* (VRSA).

As per Table no.20, Impact of various risk factors and the emergence of multidrug resistant pathogens determines the outcome of the disease. Outcome of the study is as follows:

- In CAP, 40(26.66%) lost follow up, 95 (63.33%) recovered, 15(10%) expired. Mortality rate in CAP was about 50% in the study done by A.P.Misra et al [96]
- In HAP, 5(16.66%) lost follow up, 18(60%) recovered and 7(23.33%) expired. Mortality rate is contrast to the study of Hina Gadani et al[97] 41.20%.
- In VAP, 13(52%) recovered, 12(48%) expired. Mortality rate in VAP coincide with the study of Neelima Ranjan Uma et al[91] 48.33%, S Qureshi et al[8] 48.57%. Mortality rate in VAP is mostly due to multidrug resistant pathogen.

As Pneumonia is one of the major causes of morbidity and mortality in hospital as well as in community due to varied etiopathogenesis, it is necessary to do culture of respiratory samples (sputum and ETA) in order to identify the pathogen thereby to know the antibiotic sensitivity pattern. In view of the changing patterns of bacterial resistance to common drugs, the administration of antibiotics must be judicious to prevent the emergence of bacterial resistance in the hospital and the community.

SUMMARY

Pneumonia is a disease known to mankind from antiquity. Respiratory samples (Sputum, endotracheal aspirate) and blood samples were collected from patients with clinically suspected and radiological evidence of pneumonia in adult patients, attending medicine OP and also admitted in IMCU and other wards at Government Kilpauk Medical College and Hospital, Chennai from January 2015 to June 2016.

Patients with age group of 40-60 years were commonly affected in CAP and VAP, whereas in HAP age group above 60 years were commonly affected. Males are commonly affected by pneumonia in this study. The culture positives with respiratory samples of patient with pneumonia were 83(55.33%) in CAP, 26(86.66%) in HAP, 25(100%) in VAP. Gram negative bacilli were the predominant bacteria isolated with 68(71.57%) in CAP, 24(75%) in HAP, 20(76.92%) in VAP. The culture positives with blood samples of patient with pneumonia were 8 (10%) in CAP, 2(16.66%) in HAP, 5(20%) in VAP.

Klebsiella pneumoniae was the major isolate constituting about 42 (44.21%) in CAP followed by *Staphylococcus aureus* constituting about 19 (20%) in CAP. In HAP, 12 (37.50%) were *Klebsiella pneumoniae* followed by *Escherichia coli 5 (15.62%) & Staphylococcus aureus* 4(12.50%). In VAP, 6 (23.07%) were *Klebsiella pneumoniae and Staphylococcus aureus* followed by *Pseudomonas aeruginosa& Acinetobacter baumanii* which was about 12(19.23%).

In CAP, *Klebsiella pneumoniae* showed 100% sensitive to Meropenem, 97.61% sensitive to Piperacillin –Tazobactam and Imipenem, 66.66 % sensitive to 3rd generation Cephalosporins (Cefotaxime, Ceftazidime) and Amikacin (61.90%) with least sensitive for Cotrimoxazole (11.90%) and Amoxycillin (11.90%).

In HAP, *Klebsiella pneumonia*e showed 100% sensitive to Imipenem, Meropenem, 91% sensitive to Piperacillin/Tazobactam, 83.30% sensitive to cefoxitin, 50% sensitive to 3rd generation Cephalosporins (Cefotaxime, Ceftazidime) with least sensitive for Cotrimoxazole (16.6%), cephalexin (0) and Amoxycillin (8.30%).

In VAP*, Klebsiella pneumonia*e showed 100% sensitive to Meropenem, 83.30% sensitive to cefoxitin, Piperacillin –Tazobactam, Imipenem, 50% sensitive to 3rd generation Cephalosporins (Cefotaxime, Ceftazidime) with least sensitive to Gentamicin (16.60%) and Amoxycillin (16.60%).

In CAP, *Staphylococcus aureus* showed 84.20% sensitive to Cefoxitin, Cefotaxime and Ofloxacin, least sensitive to Cotrimoxazole (26.30%), Amoxycillin (10.50%) and resistant to Cephalexin.

In HAP, *Staphylococcus aureus* showed 75% sensitive to Cefoxitin, Cefotaxime, Ofloxacin, Amikacin, Doxycycline, least sensitive to Amoxycillin (25%) and resistant to Cephalexin.

In VAP, *Staphylococcus aureus* showed 66.66% sensitive to Cefoxitin, Cefotaxime, Ofloxacin, Amikacin, least sensitive to Cotrimoxazole (16.66%), Amoxycillin (16.66%) and resistant to Cephalexin.

Among the *Klebsiella pneumoniae,* distribution of ESBL 11(26.19%) in CAP, 4(33.33%) in HAP, 3(50%) in VAP identified by phenotypic method & CTX-M is the most common gene identified by genotypic method.

Among *Pseudomonas aeruginosa,* MBL producers were detected by isolates showing resistant to Meropenem. Distribution of MBL producing *Pseudomonas aeruginosa* were 1(8.3%) in CAP, 2 (40%) in VAP.

Distribution of Amp-C producing Enterobacteriacea in pneumonia showing resistance to Cefoxitin (Zone size <18mm) were 1 (2.3%) *Klebsiella pneumoniae* and 1 (20%) *Escherichia coli* in CAP, *V*AP showed 1(16.66%) were *Klebsiella pneumoniae* and 1(25%) were *Escherichia coli.*

Distribution of MRSA in Pneumonia patients were 3(15.78%) in CAP, 1(25%) in HAP and 2(33.33%) in VAP. All isolates of MRSA showed MIC value to Vancomycin <2µg/ml, so it rules out Vancomycin Resistant *Staphylococcus aureus* (VRSA).mec A gene was positive for all the 6 isolates of MRSA.

Outcome of the study in pneumonia patients were among 40 (26.66%) lost follow up, 95 (63.33%) recovered, 15(10%) expired in CAP. In HAP 5 (16.66%) lost follow up, 18(60%) recovered and 7(23.33%) expired. In VAP 13(52%) recovered, 12(48%) expired.

Early diagnosis of pneumonia and administration of specific antibiotic therapy, based on the antibiogram of the isolated pathogen will reduce the complication as well as the emergence of drug resistance, thereby hasten the recovery of the patients. This will lead to better outcome and effective patient care.

CONCLUSION

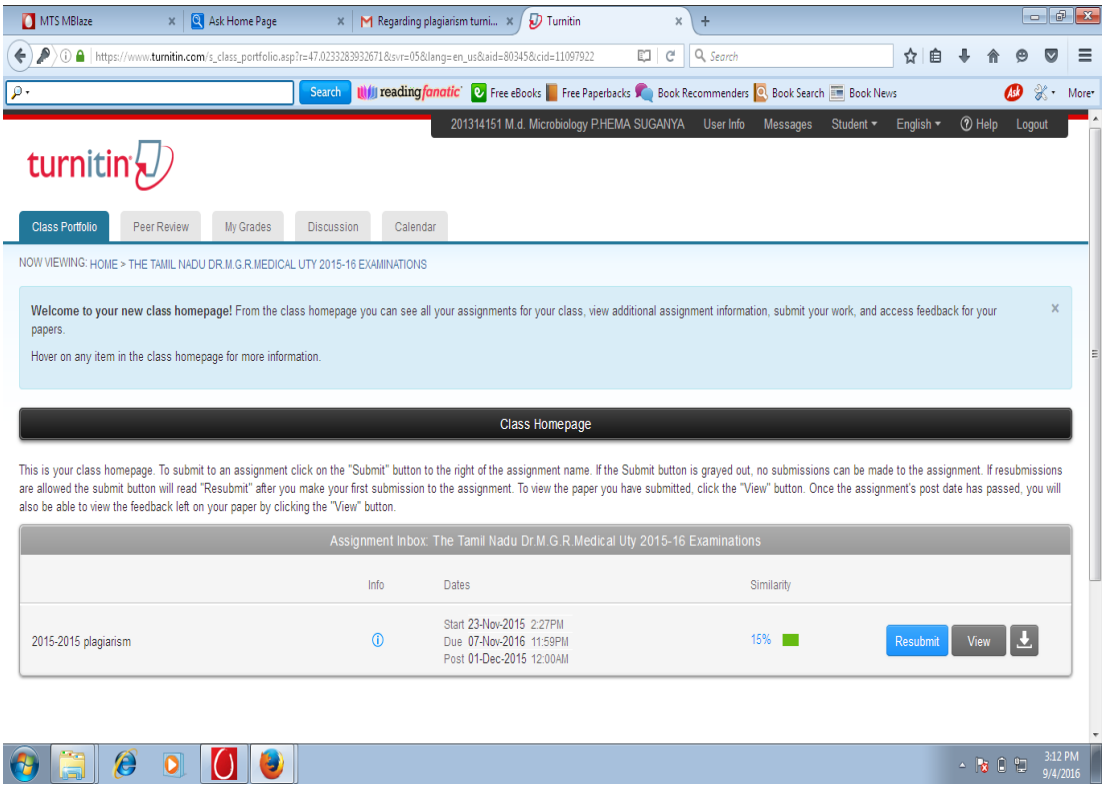
Pneumonia remains a common and serious disease with significant morbidity and mortality in developing as well as in developed nation.

- The present study showed that Gram negative bacilli were the predominant bacteria isolated from Pneumonia in adult patients.

- Among the Gram negative bacilli, *Klebsiella pneumoniae* was found to be the most common bacterial pathogen isolated with many ESBL producing strains which were 100% sensitivity to Meropenem. CTX-M was the most common gene identified among the ESBL isolates by molecular method.

- Among Gram positive organisms, *Staphylococcus aureus* was the predominant isolate. MRSA strains were detected, which showed the presence of mec A gene and all the strains were sensitive to Vancomycin (MIC E-test).

Early diagnosis of the disease with specific knowledge of the causative agents along with their isolation and sensitivity pattern will serve as an vital tool for reducing the morbidity and mortality of pneumonia. Strict adherence to infection control policy and antibiotic stewardship will reduce the emergence of drug resistant strains in pneumonia leading to faster recovery in better health care facilities.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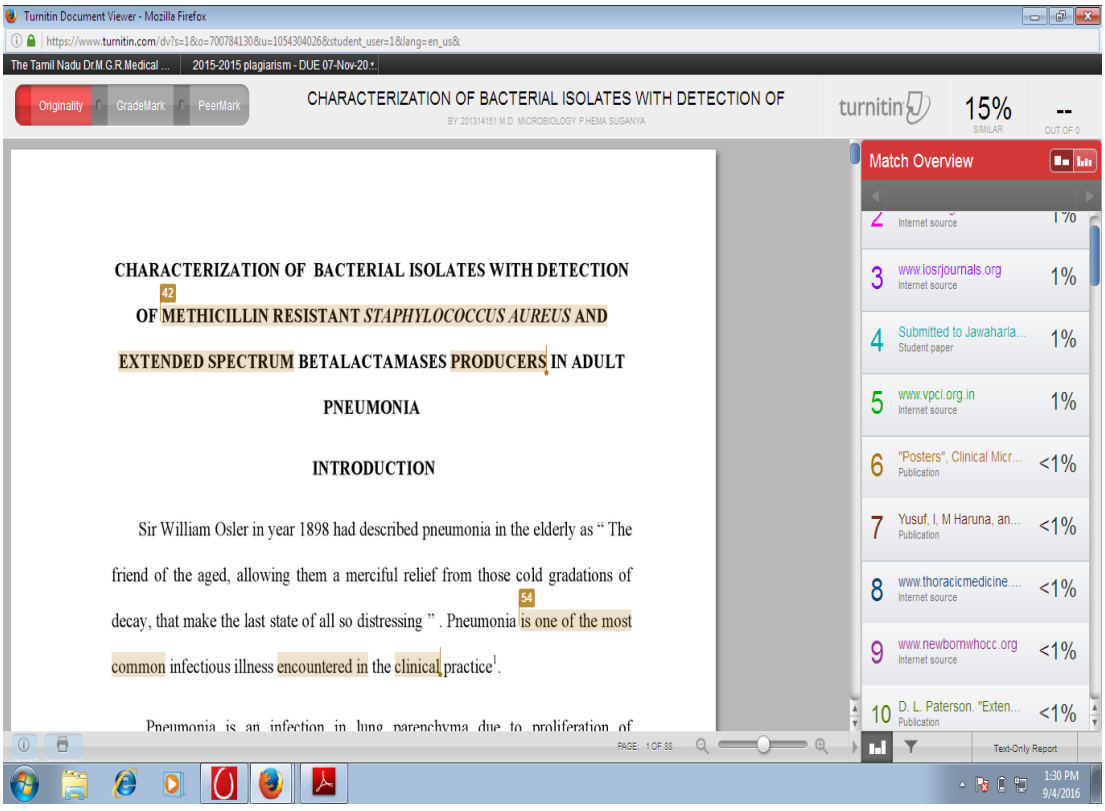
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CHARACTERIZATION OF BACTERIAL ISOLATES WITH DETECTION OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS AND EXTENDED SPECTRUM BETALACTAMASES PRODUCERS IN ADULT PNEUMONIA
INTRODUCTION
Sir William Osler in year 1898 had described pneumonia in the elderly as " The friend of the aged, allowing them a merciful relief from those cold gradations of decay, that make the last state of all so distressing " . Pneumonia is one of the most common infectious illness encountered in the clinical practice[1].
Pneumonia is an infection in lung parenchyma due to proliferation of
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CERTIFICATE OF APPROVAL

The Institutional Ethical Committee of Govt. Kilpauk Medical College, Chennai reviewed and discussed the application for approval "A Study on characterization of bacterial isolates with detection of Methicillin Resistant Staphylococcus aureus and Extended Spectrum Betalactamase producers in Adult Pneumonia" – For Project Work Submitted by Dr.P.HemaSuganya, MD (Microbiology), PG Student, KMC, Chennai – 10.

The Proposal is APPROVED.

The Institutional Ethical Committee expects to be informed about the progress of the study any Adverse Drug Reaction Occurring in the Course of the study any change in the protocol and patient information /informed consent and asks to be provided a copy of the final report.

DEAN,
Govt.Kilpauk Medical College,
Chennai – 10.

My Comp<E Colon< ME 1 Sec> Ethical Committee

PROFORMA

Name: OP/IPno:

Age/Sex: Ward:

Occupation: Micro.lab no:

Address: Date:

Complaints:

Comorbidity:

H/o Antibiotics/ Hospitalisation(duration):

H/o smoking / alcohol intake:

If patient on ventilator (duration):

Clinical examination:

Vital signs: PR- RR- Temp- BP- Spo2-

Clinical diagnosis-

Diagnosis based on classification-

Investigation:

Total WBC count-

Chest X-ray:

Microbiology investigation:

Samples collected-

1.Sputum for AFB-

2.Culture &Sensitivity (Sputum/Endotracheal aspirate)

Macroscopic examination-

Microscopic examination-

Direct Gram stain-

Bacterial Culture-

Organism-

Antibiotic Sensitivity-

ESBL/MRSA/MBL/AMP C-

2.Blood culture &Sensitivity-

Culture-

Organism-

Antibiotic Sensitivity-

ESBL/MRSA/MBL/AMP C-

OUTCOME-

சுய ஒப்புதல் படிவம்

ஆய்வு செய்யப்படும் தலைப்பு: "CHARACTERISATION OF BACTERIAL ISOLATES WITH DETECTION OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* AND EXTENDED SPECTRUM BETALACTAMASE PRODUCERS IN ADULT PNEUMONIA" .

ஆய்வு செய்யப்படும் இடம்: கீழ்பாக்கம் அரசு மருத்துவக்கல்லூரி மற்றும் மருத்துவமனை, சென்னை-10

பங்கு பெறுபவரின் பெயர்:

பங்கு பெறுபவரின் வயது: பங்குபெறுபவரின் எண் :

மேலே குறிப்பிட்டுள்ள மருத்துவ ஆய்வின் விவரங்கள் எனக்கு விளக்கபட்டுள்ளது. நான் இவ்வாய்வில் தன்னிச்சையாக பங்கேற்கின்றேன். எந்த காரணத்தினாலோ, எந்த சட்ட சிக்கலுக்கும் உட்படாமல் நான் இவ்வாய்வில் இருந்து விலகிக் கொள்ளலாம் என்றும் அறிந்துகொண்டேன்.

இந்த ஆய்வு சம்பந்தமாகவோ, இதை சார்ந்து மேலும் ஆய்வு மேற்கொள்ளும் போதும் இந்த ஆய்வில் பங்குபெறும் மருத்துவர் என்னுடைய மருத்துவர் என்னுடைய மருத்துவ அறிக்கைகளை பார்ப்பதற்கு என் அனுமதி தேவை இல்லை என அறிந்து கொள்கிறேன். இந்த ஆய்வின் மூலம் கிடைக்கும் தகவலையோ,முடிவையோ பயன்படுத்திக் கொள்ள மறுக்கமாட்டேன்.

இந்த ஆய்வில் பங்கு கொள்ள ஒப்புக் கொள்கிறேன். இந்த ஆய்வை மேற்கொள்ளும் மருத்துவ அணிக்கு உண்மையுடன் இருப்பேன் என்று உறுதியளிக்கிறேன்.

பங்கேற்பவரின் கையொப்பம்: சாட்சியாளரின் கையொப்பம்

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தேதி: தேதி :

பங்கேற்பவரின் பெயர் மற்றும் விலாசம்:

ஆய்வாளரின் கையொப்பம்:

இடம்: தேதி:

PATIENT CONSENT FORM

STUDY DETAIL: "**CHARACTERISATION OF BACTERIAL ISOLATES WITH DETECTION OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* AND EXTENDED SPECTRUM BETALACTAMASE PRODUCERS IN ADULT PNEUMONIA''.**

STUDY CENTER:
KILPAUK MEDICAL COLLEGE AND HOSPITAL, CHENNAI.
PATIENT NAME: PATIENT AGE:
IDENTIFICATION NUMBER:
PATIENT TO TICK THESE BOXES

I conform that I have understood the purpose of procedure for the above study.

I have the opportunity to ask the question and all my questions and doubts have been answered to my satisfaction.

I understand that my participation in the study is voluntary and that I am free to withdraw at anytime without giving any reasons, without my legal rights being affected.

I understand that investigator, regulatory authorities and the ethics committee will not need my permission to look at my health records both in respect to the current study and any further research that may be conducted in relation to it, even if withdraw from the study, I understand that my identity will not be revealed in any information released to third parties or published, unless as required under the law. I agree not to restrict the use of any data or results that arise from the study.

I agree to take part in the above study and to comply with the instructions given during the study and faithfully cooperative with the study team and to immediately inform the study staff if I suffer from any deterioration in my health or wellbeing or any unexpected or unusual symptoms.

I hereby give consent to participate in this study.

I hereby give permission to undergo complete clinical examination and diagnostic test.
Signature/Thumb impression: Place: Date:
Patient name and address:

Signature of the investigator: Place: Date:
Study investigator's name:

APPENDIX

GRAM STAIN

Primary stain	crystal violet 10g
	Absolute alcohol 100ml
	Distilled water 1 litre
Mordant	Grams iodine 10g
	Potassium iodide 20g
	Distilled water-1litre
Decolouriser	Acetone
Counter stain-	Dilute Carbol fuchsin

1. Flood the crystal violet for one minute
2. Rinse gently with distilled water
3. Flood the slide with grams iodine for one minute
4. Rinse gently with distilled water
5. Decolourise with acetone for only 2-3 secs
6. Rinse gently with distilled water to remove excess of decolouriser
7. Flood the slide with dilute carbol fuchsin for one minute
8. Rinse the slide with distilled water air dry and examine under oil immersion objective

PEPTONE WATER

Peptone	10g
Sodium chloride	5g
Distilled water	1 litre

Dissolve the ingredients in warm water, adjust the pH to 7.4 - 7.5 and filter.

Distribute as required and autoclave at 121 degree Celsius for 15 mins.

Brain Heart Infusion broth:-

Calf brain infusion	200g
Beef heart infusion	250g
Proteose peptone	10g
Dextrose	2g
Sodium chloride	5g
Disodium phosphate	2.5g
Distilled water	1000ml

Add the contents and dissolve by heating.

Adjust the pH to 7.4 + 0.2. Autoclave at 121ºC for 15 minutes.

MacConkey agar:-

This is a useful medium for the cultivation of Enterobacteriacea. It contains a bile salt to inhibit non-intestinal bacteria and lactose with neutral red to distinguish the lactose-fermenting coli forms from the lactose –non-fermenting *Salmonella* and *Shigella* groups. The concentration of sodium taurocholate may be reduced to suit less tolerant organisms. The omission of sodium chloride from the medium prevents the spreading of *Proteus* colonies.

Peptone	20 g
Sodium taurocholate	5 g
Water	1 litre
Agar	20 g
Neutral red solution, 2% in 50% ethanol	3.5 ml
Lactose, 10% aqueous solution	100 ml

Dissolve the peptone and taurocholate (bile salt) in the water by heating. Add the agar and dissolve it in the steamer or autoclave. If necessary, clear by filtration. Adjust the pH to 7.5. Add the lactose and the neutral red, which should be well shaken before use, and mix. Heat in the autoclave with 'free steam' (100^{o} C) for 1hr, then at 115^{o} C for 15 min. poured in plates.

Nutrient agar:-

Peptic digest of animal tissue	5g
Beef extract	1.5g
Yeast extract	5g
Agar	15g
Distilled water	1000ml

Dissolve the contents in water and mix by heating Autoclave at 121° C for 15 minutes. Adjust pH to 7.4 ± 0.2. Pour 20-25 ml of 9 cm diameter. Petri dishes to give 4 mm thickness.

Blood agar:-

Sterile sheep blood	50 ml
Peptone	10 g
Beef extract	3g
Sodium chloride	5 g
Distilled water	1000 ml

Autoclave the nutrient agar base at 121° C for 15 minutes and cooled to 50° C. Then the blood is added with sterile precautions and distribute in Petri dishes.

Chocolate agar:-

Sterile sheep blood	50ml
Peptone	10 g
Beef extract	3g
Sodium chloride	5 g
Distilled water	1000 ml

Autoclave the nutrient agar base at 121° C for 15 minutes and allow the medium to be at 75° C. Then the blood is added with sterile precautions with gentle agitation from time to time and the colour of the medium is chocolate brown. Distribute in Petri dishes.

Mueller Hinton agar:-

Beef infusion	300 ml
Casein hydrolysate	17 gm
Starch	1.5 gm
Agar	10 gm
Distilled water	1000 ml

Emulsify the starch in a small amount of cold water, pour into the beef infusion and add the casein- hydrolysate and the agar. Make up the volume to 1000 ml (1 litre) with distilled water. Dissolve the constituents by heating gently at 100° C with agitation.

Adjust the pH to 7.4. Dispense in screw-capped bottles and sterilize by autoclaving at 121 ^{o} C for 20 minutes. 20 to 25 ml of it is poured into Petri dishes of 9 cm diameter to give a thickness of 4mm.

McFarland Turbidity Standards for inoculum preparation

A Barium sulphate 0.5 McFarland standards was prepared as follows

1. A 0.5 ml of 0.048mol/L of Barium chloride was added to 99.5 ml of 0.18 mol/L of H2SO4 with constant stirring to maintain a suspension.
2. Correct density of the turbidity standard was verified by using a spectrophotometer. The absorbance of 625nm should be 0.08 to0.10 for the 0.5 McFarland standards.
3. The Barium sulphate suspension was transferred in 4-6 ml to a screw capped tube of the same size as those used in growing or diluting the bacterial inoculum.
4. These tubes were tightly sealed and stored in the dark at room temperature.
5. The Barium sulphate turbidity standard was vigorously agitated before each use and inspected for a uniform turbid appearance

Differentiating Characters of Isolates Commonly Observed In Pneumonia patients Among Gram positive cocci

Garam strain	Catalase	Modified Oxidase	Heamolysis	Coagulase	Mannitol	Bile esculin agar	Bacitracin	Optochin	Isolate
Cocci in cluster	Positive	Negative	±	Positive	Positive	Negative	Resis tant	Negative	*Staphylococcus aureus*
Cocci in cluster	Positive	Negative	±	Negative	Negative	Negative	Resis tant	Negative	*Coagulase negative Staphylococcus aureus*
Cocci in pairs	Negative	Negative	α - hemolysis	Negative	Negative	Negative	Resis tant	Positive	*Streptococcus pneumoniae*
Cocci in pairs	Negative	Negative	±	Negative	±	Positive	Resis tant	Negative	*Enterococci*

Differentiating Characters Of Isolates Commonly Observed In Pneumonia patients Among Gram Negative Bacilli

Organism	TSI	Citrate	Indole	Oxidase	Catalase	Glucose	Lactose	Sucrose	Maltose	Mannose	Motility
Klebsiella Pneumoniae	A/A with gas	Utilised	-	-	+	+	+	+	+	+	-
Escherichia coli	A/A with gas	Not utilised	+	-	+	+	+	-	+	+	+
Pseudomonas aeruginosa	K/No change	Utilised	-	+	+	-	-	-	±	-	+
Proteus sp. species	K/A with H2S	Utilised	±	-	+	+	-	+	-	-	+
Serratia marcesens	K/A	Utilised	-	-	+	+	-	+	-	-	+
Acinetobacter species	K/No Change	Not utilised	-	-	+	-	-	-	-	-	-

Note: A/A = Acid slant / Acid butt, K/A = Alkaline slant / Acid butt,

+ = Positive, - = Negative

ZONE SIZE INTERPRETATIVE CHART IN ACCORDING TO CLSI - 2015 Kirby-Bauer Chart

Sl. No.	Drug	Disk Content µg	Resistant mm or less	Intermediate mm	Sensitive mm or more
1.	Amoxycillin	10 mcg	14 mm	15-16 mm	17 mm
2.	Erythromycin	15	≤ 13	14-22	≥ 23
3.	Doxycycline	30	≤ 12	13-15	≥ 16
4.	Cephalexin	30	≤ 14	15-17	≥ 18
5.	Ofloxacin	5	≤ 12	13-15	≥ 16
6.	Ciprofloxacin	5	≤ 15	16-20	≥ 21
7.	Gentamycin	10	≤ 12	13-14	≥ 15
8.	Cefotaxime	30	≤ 22	23-25	≥ 26
9.	Ceftazidime	30	≤ 17	18-20	≥ 21
10.	Linezolid	30	-	-	≥ 21
11.	Amikacin	30	≤14	15-16	≥ 17
12.	Imipenem	10	≤ 19	20-22	≥ 23
13.	Piperacillin/tazobactum	100/10	≤ 17	18-20	≥ 21

ABBREVIATION

CAP	-	Community Acquired Pneumonia
HAP	-	Hospital Acquired Pneumonia
VAP	-	Ventilator Associated Pneumonia
ETA	-	Endotracheal Aspirate
CPIS	-	Clinical Pulmonary Infection Score
PMN	-	Polymorphonuclear cells
ATCC	-	American Type Culture Collection
CLSI	-	Central Laboratory Standards Institute
GNB	-	Gram Negative Bacilli
GPC	-	Gram Positive Cocci
CFU	-	Colony Forming Units.
ESBL	-	Extended Spectrum Beta Lactamase
E- Test	-	Epsilometer Test
MHA	-	Mueller Hinton Agar
MIC	-	Minimum Inhibition Concentration
PCR	-	Polymerase Chain Reaction
PBP	-	Pencillin Binding Protein
SHV	-	Sulphy Hydryl Variable
TEM	-	Temoniera
MRSA	-	Methicillin Resistant *Staphylococcus aureus*
ESBL	-	Extended Spectrum Beta Lactamases
MBL	-	Metallo Beta Lactamases
Amp C	-	Amp C producing Beta Lactamases
SPSS	-	Stastical Package for the Social Science

KEY TO MASTER CHART

OP No	-	Outpatient number
IP No	-	Inpatient number
CAP	-	Community Acquired Pneumonia
HAP	-	Hospital Acquired Pneumonia
VAP	-	Ventilator Associated Pneumonia

Samples:

SP	-	Sputum
B	-	Blood
E	-	Endotracheal aspirate
CNP	-	Culture negative for pathogen
NG	-	No growth
GNB	-	Gram Negative Bacilli
GPC	-	Gram Positive Cocci
BS	-	Barlett scoring
P G10 U	-	Penicillin G 10 Units
AMX	-	Amoxycillin
AMC	-	Amoxycillin + Clavulanic acid
DO	-	Doxycycline
E	-	Erythromycin
CN	-	Cephalexin
CTX	-	Cefotaxime
CAZ	-	Ceftazidime
CTR	-	Ceftriaxone
AK	-	Amikacin
GEN	-	Gentamicin
OF	-	Ofloxacin
CIP	-	Ciprofloxacin

COT	-	Cotrimoxazole
PTZ	-	Piperacillin + Tazobactum
IMP	-	Imipenem
MRP	-	Meropenem
VAN	-	Vancomycin
CX	-	Cefoxitin
S	-	Sensitive
R	-	Resistant
MRSA	-	Methicillin Resistant *Staphylococcus aureus*
ESBL	-	Extended Spectrum Beta Lactamases
MBL	-	Metallo Beta Lactamases producers
Amp C	-	Amp C producing Beta Lactamases

Outcome:

EX	-	Expired
RD	-	Recovered
LF	-	Lost follow up

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S.No	Age	Sex	OP/IP No	Ward	Comorbidity	Antibiotic	Hospitalisation Without ventilation (>48hrs)	Mechanical Ventilation		Smoker	Aalddss	Classification	Chest X - ray	Samples	Direct Gram Stain	Organism	AMX	P G10 U	AMC	E	DO	CN	CTX	CTR	CAZ	AK	GEN	OF	CIP	COT	PTZ	IMP	MRP	VAN	CX	MRSA	ESBL	AMP C	MBL	OUTCOME
								3-5 days	>5																															
1	36	M	78455	OP	–	–	–	–	–	yes	yes	CAP	RLL	SP	Few pus cells &no organism	CNP																									LF
														B		NG																							
2	24	F	78510	OP	–	–	–	–	–	no	no	CAP	LUL	SP	Few pus cells &no organism	CNP																									LF
														B		NG																							
3	42	M	8670	IM CU	COP D	yes	yes	–	–	no	no	HAP	R ML	SP	BS +1,pus cells,GPC &GNB	K.pneumoniae	R		S				R	R	R	S	R		R	R	S	S	S		R			AM P C		RD
															BS +1,pus cells,GPC pairs	E.faecalis	R		S	S	S					S	S(HL G120)		S	R				S					
														B		NG																							
4	62	M	78555	OP	DM /HT	–	–	–	–	yes	yes	CAP	R UL	SP	Few pus cells &no organism	CNP																									LF
														B		NG																							
5	29	M	79865	OP	–	–	–	–	–	yes	yes	CAP	R LL	SP	Few pus cells &no organism	CNP																									LF
														B		A,baumanii																							
6	63	F	79964	OP	–	–	–	–	–	no	no	CAP	B/L	SP	Few pus cells &no organism	CNP																									LF
														B		NG																							
7	68	M	80005	OP	DM /COPD	–	–	–	–	yes	yes	CAP	R LL	SP	BS +1,pus cells,GNB	*K.pneumoniae*	R		S			R	S	S	S	S	R	S	S	S	S	S	S		S					RD
														B		NG								a															
8	19	F	80022	OP	–	–	–	–	–	no	no	CAP	R LL	SP	Few pus cells &no organism	CNP																									LF
														B		NG																							
9	55	F	8969	ORTHO	DM/ #R hand	yes	yes	–	–	no	no	HAP	B/L	SP	BS +1,pus cells,GNB &GPC	K.pneumoniae	R		S				R	R	R	R	R		R	R	S	S	S		S		ESBL			EX
																s.aureus	R		R	S	S					R	S		S	S			NT	S				RD
														B		NG																							
10	22	M	80099	OP	/COPD	–	–	–	–	yes	yes	CAP	R LL	SP	BS +1,pus cells,GNB	*K.pneumoniae*	R		R			R	R	R	R	S	R	S	R	R	S	S	S		S		ESBL			LF
														B		NG																							
11	67	M	80192	OP	DM	–	–	–	–	yes	yes	CAP	L LL	SP	BS +1,pus cells,GNB	*K.pneumoniae*	R		R			R	S	S	S	R	R	S	R	S	S	S	S		R			AM P C		EX
														B		NG																							
12	35	F	80233	OP	–	–	–	–	–	no	no	CAP	R ML	SP	Few pus cells &no organism	CNP																									LF
														B		NG																							
13	33	M	9065	MW	COPD	yes	yes	–	–	yes	yes	HAP	B/L	SP	BS +1,pus cells,GNB	K.pneumoniae	R		R				S	S	S	S	S		S	S	S	S	S		S					RD
14	60	F	9265	IM CU	DM / COPD	YES	YES	yes	–	no	no	VAP	R ML	E	pus cells,GNB	K.pneumoniae	R		S				R	R	R	R	R		R	R	S	S	S		S		**ESBL**			EX
														B		K.pneumoniae	R		S			S	R	R	R	R	R		R	R	S	S	S		S					
15	68	M	80333	OP	DM /COPD	–	–	–	–	yes	yes	CAP	R LL	SP	BS +1,pus cells,GNB	P.aeruginosa	NT		S			NT	NT	S	S	S	R		R	NT	S	S	S		NT					RD
														B		P.aeruginosa	NT		S			NT	NT	S	S	S	R		R	NT	S	S	S		NT					
16	25	F	80543	OP	–	–	–	–	–	no	no	CAP	R LL	SP	Few pus cells &no organism	CNP																									LF
														B		NG																							
17	55	F	80976	OP	DM	–	–	–	–	no	no	CAP	R LL	SP	Few pus cells &no organism	CNP																									LF
														B		NG																							
18	66	M	81234	OP	DM	–	–	–	–	no	no	CAP	LUL	SP	Few pus cells &no organism	CNP																									LF
														B		NG																							

19	23	F	81287	OP	–	–	–	–	–	no	yes	CAP	LUL	SP	Few pus cells &no organism	CNP																								LF
														B		NG																								
20	65	M	81299	OP	DM/HT	–	–	–	–	yes	no	CAP	R LL	SP	BS +1,pus cells,GNB	P.aeruginosa	NT		R			NT	NT	S	S	R	R				S	S	S		NT					RD
														B		NG																								
21	71	M	82321	OP	DM	–	–	–	–	yes	yes	CAP	L LL	SP	BS +1,pus cells,GNB	*K.pneumoniae*	R		S			R	R	R	R	R	R	R	R	R	S	S	S		S		ESBL			EX
														B		NG																								
22	29	M	82354	OP	–	–	–	–	–	no	no	CAP	R ML	SP	Few pus cells &no organism	CNP																								LF
														B		NG																								
23	42	F	82399	OP	DM/HT/COPD	–	–	–	–	no	no	CAP	R LL	SP	BS +1,pus cells,GNB,GPC	*K.pneumoniae*	R		S			R	R	R	R	S	R	S	R	R	S	S	S		S		ESBL			
																S.aureus	R			R	S	R	R			S	R	S	R	R				NT	S					
														B		S.aureus	R			R	S	R	R			S	R	S	R	R				NT	S					
24	63	M	82432	OP	DM/COPD	–	–	–	–	no	no	CAP	R LL	SP	BS +1,pus cells,GNB	*K.pneumoniae*	R		R			R	R	R	R	R	R	R	R	R	S	S	S		S		ESBL			RD
																CONS	R			S	S	R	S			S	R	S	S	R				NT	S					
25	30	M	82445	OP	–	–	–	–	–	no	no	CAP	LUL	SP	Few pus cells &no organism	CNP																								LF
														B		NG																								
26	55	F	82465	OP	–	–	–	–	–	no	no	CAP	B/L	SP	Few pus cells &no organism	CNP																								LF
														B		NG																								
27	38	M	82876	OP	COPD	–	–	–	–	no	yes	CAP	B/L	SP	Few pus cells &no organism	S.marcesens	R		S			R	S	S	S	S	R	S	S	R	S	S	S		NT					RD
28	64	M	82912	OP	DM	–	–	–	–	no	no	CAP	R LL	SP	BS +1,pus cells,GNB	P.aeroginosa	NT		S			NT	NT	S	S	S	S				S	S	S		NT					RD
29	21	F	82934	OP	–	–	–	–	–	no	no	CAP	L LL	SP	Few pus cells &no organism	CNP																								LF
														B		NG																								
30	26	F	82965	OP	–	–	–	–	–	no	no	CAP	R LL	SP	Few pus cells &no organism	CNP																								LF
														B		NG																								
31	49	M	82999	OP	DM	–	–	–	–	no	no	CAP	R LL	SP	Few pus cells &no organism	CNP																								LF
														B		NG																								
32	19	M	83234	OP	–	–	–	–	–	yes	no	CAP	R LL	SP	Few pus cells &no organism	CNP																								LF
														B		NG																								
33	58	M	83256	OP	DM/HT/COPD	–	–	–	–	yes	yes	CAP	R LL	SP	BS +1,pus cells,GNB	*K.pneumoniae*	R		R			R	R	R	R	S	R	S	R	R	S	S	S		S		ESBL			RD
34	41	M	83324	OP	DM	–	–	–	–	yes	yes	CAP	L LL	SP	BS +1,pus cells,GNB	*K.pneumoniae*	R		R			R	R	R	R	R	R	R	R	R	S	S	S		S		ESBL			LF
35	65	M	9254	SW	DM/HT/anal fissure	yes	yes	–	–	yes	yes	HAP	R LL	SP	BS +1,pus cells,GNB	P.aeroginosa	R		R				S	S	S	S	R		R		S	S	S		S					RD
36	59	M	83387	OP	DM	–	–	–	–	yes	yes	CAP	R LL	SP	BS +1,pus cells,GNB	*K.pneumoniae*	R		R			R	R	R	R	R	R	R	R	R	S	S	S		S		ESBL			RD
														B		NG																								
37	27	F	83432	OP	–	–	–	–	–	no	no	CAP	R LL	SP	Few pus cells &no organism	CNP																								LF
														B		NG																								
38	68	M	83486	OP	DM/COPD	–	–	–	–	yes	yes	CAP	R LL	SP	Few pus cells &no organism	*S.aureus*	R			R	S	R			S	S	S	S	S	S				NT	S					EX
39	68	M	11643	IMCU	DM/HT/CKD	YES	YES	yes	–	yes	yes	VAP	L LL	E	pus cells,GPC clusters	S.aureus	R			R	S					R	R		R	R				NT	S					RD
														B		NG																								
40	28	M	83513	OP	–	–	–	–	–	yes	yes	CAP	R LL	SP	Few pus cells &no organism	CNP																								LF

														B		NG																										
41	61	F	83542	OP	DM	–	–	–	–	no	no	CAP	R LL	SP	Few pus cells &no organism	*CONS*	R			S	S	R	S	S					R	S	S	R				NT	S					RD
42	32	M	83562	OP	–	–	–	–	–	yes	yes	CAP	R LL	SP	Few pus cells &no organism	CNP																										LF
														B		NG																										
43	29	F	83578	OP	–	–	–	–	–	yes	yes	CAP	R LL	SP	Bs +1,pus cells,GPC IN PAIRS	S.pneumoniae	R	S		S	S	R	S			S		S	R	S	R	S				NT						RD
44	63	M	9698	IMCU	DM /AKI	yes	yes	–	–	yes	yes	HAP	B/L	SP	Bs +1,pus cells,GNCB	K.pneumonia	R		R				S	S	S	S	S	S	S		S	R	S	S	S		S					RD
45	58	M	11824	IMCU	DM/ HT/ CKD	YES	YES	–	yes	yes	yes	VAP	B/L	E	pus cells,GNCB	A,baumanii	R		R				S	S	S	S	S	S	R		R	S	S	S	S		S					RD
														B		NG																										
46	61	M	83589	OP	–	–	–	–	–	yes	yes	CAP	L LL	SP	Few pus cells &no organism	CNP																										LF
														B		NG																										
47	36	M	83599	OP	COPD	–	–	–	–	yes	yes	CAP	R LL	SP	Bs +1,pus cells,GNB	P.aeroginosa	NT		R			NT	NT	S	S	S	S	S	S				S	S	S		NT					RD
48	37	F	83632	OP	–	–	–	–	–	no	no	CAP	R ML	SP	Few pus cells &no organism	CNP																										LF
														B		NG																										
49	21	M	83665	OP	–	–	–	–	–	yes	yes	CAP	L LL	SP	Few pus cells &no organism	CNP																										LF
														B		NG																										
50	61	F	83675	OP	DM	–	–	–	–	no	no	CAP	R LL	SP	Bs +1,pus cells,GNB	P.aeroginosa	NT		R			NT	NT	R	R	R	R	R	S	R	S		S	R	R		NT		MBL			RD
51	59	M	83689	OP	DM /COPD	–	–	–	–	yes	yes	CAP	R LL	SP	Bs +1,pus cells,GNB	*K.pneumoniae*	R		S			R	R	R	R	R	R	R	R	S	R	R	S	S	S		S		ESBL			RD
																E.coli	R		S			R	S	S	S	S	S	S	S	R	S	R	S	S	S		S					
52	36	M	83698	OP		–	–	–	–	yes	yes	CAP	R LL	SP	Bs +1,pus cells,GPC CLUSTERS	*S.aureus*	R			S	S	R			S	S	S	S	S	S	S	S				NT	S					RD
53	26	M	83702	OP	–	–	–	–	–	yes	yes	CAP	R LL	SP	Few pus cells &no organism	CNP																										LF
														B		NG																										
54	68	M	10321	MW	ALD	yes	yes	–	–	yes	yes	HAP	L LL	SP	Bs +1,pus cells,GNB	S.marcesecens	R		R				S	S	S	S	S	S	R		R	S	S	S								RD
55	65	F	83225	OP	DM	–	–	–	–	no	no	CAP	R ML	SP	BS +1,pus cells,GNB	*K.pneumoniae*	R		S			R	R	R	R	S	R	S	R	R	R	R	S	S	S		S		ESBL			EX
																E.coli	R		R			R	S	S	S	S	S	S	S	R	S	R	S	S	S		S					
56	23	M	83298	OP	–	–	–	–	–	yes	yes	CAP	R LL	SP	Few pus cells &no organism	CNP																										LF
														B		NG																										
57	57	M	83345	OP	DM	–	–	–	–	yes	yes	CAP	B/L	SP	Bs +1,pus cells,GNB	P.aeroginosa	NT		R			NT	NT	S	S	R	S	R	R				S	S	S		NT					RD
58	28	M	83356	OP	–	–	–	–	–	no	no	CAP	R LL	SP	Few pus cells &no organism	CNP																										LF
														B		NG																										
59	59	M	83457	OP	DM	–	–	–	–	no	yes	CAP	R LL	SP	Bs +1,pus cells,GPC CLUSTERS	*S.aureus*	S			S	S	R			S	S	S	S	S	S	S	S				NT	S					RD
														B		*S.aureus*	S			S	S	R			S	S	S	S	S	S	S	S				NT	S					
60	50	F	83567	OP	DM/ HT	–	–	–	–	no	no	CAP	R LL	SP	Few pus cells &no organism	CNP																										LF
														B		NG																										
61	50	M	83598	OP	DM /HT	–	–	–	–	no	yes	CAP	L LL	SP	Few pus cells &no organism	CNP																										LF
														B		NG																										

62	45	M	83678	OP	DM	–	–	–	–	no	yes	CAP	R LL	SP	Bs +1,pus cells,GNB	*K.pneumoniae*	R		R			R	R	R	R	R	R	S	R	R	S	S	S		S		ESBL			RD
																E.coli	R		S			R	S	S	S	S	R	S	R	R	S	S	S		S					
63	43	M	83690	OP	DM	–	–	–	–	yes	no	CAP	R LL	SP	Bs +1,pus cells,GNCB	*K.pneumoniae*	S		R			S	S	S	S	S	S	S	R	R	S	S	S		S					RD
														B		*K.pneumoniae*	S		R			S	S	S	S	S	S	S	R	R	S	S	S		S					
64	65	F	83721	OP	DM /COPD	–	–	–	–	no	no	CAP	R LL	SP	Bs +1,pus cells,GNCB,GPC	*K.pneumoniae*	R		R			R	S	S	S	S	R	S	R	R	S	S	S		S					RD
																S.aureus	R			S	S	R	S			S	R	S	R	R				NT	S					
														B		*K.pneumoniae*	R		R			R	S	S	S	S	R	S	R	R	S	S	S		S					
65	62	M	83765	OP	DM	–	–	–	–	yes	no	CAP	R LL	SP	Few pus cells &no organism	*K.pneumoniae*	R		S			S	S	S	S	S	R	S	R	R	S	S	S		S					EX
														B		*K.pneumoniae*	R		R			R	S	S	S	S	R	S	R	R	S	S	S		S					
66	68	M	83796	OP	DM /COPD	–	–	–	–	yes	no	CAP	R LL	SP	BS +1,pus cells,GNB	*K.pneumoniae*	S		S			S	S	S	S	S	S	S	S	S	S	S	S		S					RD
67	28	F	10428	OG	ANC	yes	yes	–	–	no	no	HAP	L DIFFUSE	SP	BS +1,pus cells,GPC	S.aureus	R			R	R	R	R			S	R	R	R	R				NT	R	**MRSA**				RD
														B		S.aureus	R			S	S	R	S			S	R	S	S	S				NT	S					
68	54	M	83845	OP	DM /HT	–	–	–	–	no	no	CAP	R ML	SP	Few pus cells &no organism	CNP																								LF
														B		NG																								
69	64	M	84321	OP	DM	–	–	–	–	no	no	CAP	R LL	SP	BS +1,pus cells,GNB	*K.pneumoniae*	R		R			S	S	S	S	S	R	S	S	R	S	S	S		S					EX
70	54	M	84398	OP	DM /HT	–	–	–	–	no	no	CAP	L LL	SP	Few pus cells &no organism	CNP																								LF
														B		NG																								
71	59	F	84435	OP	DM	–	–	–	–	no	no	CAP	R UL	SP	BS +1,pus cells,GNB,GPC	P.aeroginosa	NT		R			NT	NT	S	S	S	R	S	R		S	S	S		NT					RD
																CONS	R			S	S	R	S			S	R	S	R	R				NT	S					
72	58	M	84465	OP	DM /HT	–	–	–	–	no	no	CAP	R LL	SP	BS +1,pus cells,GNB	P.aeroginosa	NT		S			NT	NT	S	S	S	S				S	S	S		NT					RD
														B		NG																								
73	45	F	10531	OG	DM /HT/ HYSTERCTOMY	yes	yes	–	–	no	no	HAP	L LL	SP	Few pus cells &no organism	CNP																								LF
														B		NG																								
74	57	M	84497	OP	DM /COPD	–	–	–	–	yes	yes	CAP	B/L	SP	BS +1,pus cells,GPC	*S.aureus*	S			S	S	R			S	S	S	S	S	S				NT	S					RD
75	62	M	84563	OP	DM /HT	–	–	–	–	yes	yes	CAP	R LL	SP	Few pus cells &no organism	CNP																								LF
														B		NG																								
76	56	F	84598	OP	DM	–	–	–	–	no	no	CAP	LUL	SP	Few pus cells &no organism	CNP																								LF
														B		NG																								
78	55	M	84643	OP	DM /HT	–	–	–	–	yes	yes	CAP	R UL	SP	Few pus cells &no organism	CNP																								LF
														B		NG																								
79	56	M	13902	IMCU	DM/ HT/ DCLD	YES	YES	–	yes	yes	no	VAP	L DIFFUSE	E	pus cells,GPC clusters	S.aureus	R			R	R					R	R	R	R	R				NT	R	**MRSA**				EX
														B		S.aureus	R			S	S	R	S			S	S	S	R	R				NT	S					
80	67	M	84734	OP	DM	–	–	–	–	yes	yes	CAP	LUL	SP	Few pus cells &no organism	CNP																								LF
														B		NG																								
81	29	F	84756	OP	–	–	–	–	–	no	no	CAP	R LL	SP	BS +1,pus cells,GNB	*E.coli*	S		R		R	S	S	S	S	S	R	S	R	R	S	S	S		S					RD
82	65	M	84799	OP	DM /HT	–	–	–	–	yes	yes	CAP	L LL	SP	BS +1,pus cells,GPC	CONS	R			S	S	R	S			S	S	S	R	R				NT						RD
														B		CONS	R			S	S	R	S			S	S	S	R	R				NT	S					

83	54	M	84888	OP	DM /HT	–	–	–	–	yes	yes	CAP	B/L	SP	Few pus cells &no organism	CNP																								LF
														B		NG																								
84	67	M	84897	OP	DM	–	–	–	–	yes	yes	CAP	R LL	SP	BS +1,pus cells,GNB	P.aeroginosa	NT		S			NT	NT	S	S	S	S				S	S	S		NT					RD
85	63	F	84932	OP	DM	–	–	–	–	no	no	CAP	B/L	SP	Few pus cells &no organism	CNP																								LF
														B		NG																								
86	42	M	10564	ORTHO	DM / #METARSAL	yes	yes	–	–	no	no	HAP	R DIFFUSE	SP	BS +1,pus cells,GNB	E.coli	R		S				S	S	S	S	S		S	R	S	S	S		S					RD
87	41	F	84965	OP	DM	–	–	–	–	no	no	CAP	R LL	SP	BS +1,pus cells,GPC	*S.aureus*	R			S	S	R			S	S	S	S	S	R				NT	S					RD
														B		NG																								
88	68	M	84987	OP	DM /COPD	–	–	–	–	yes	yes	CAP	L LL	SP	BS +1,pus cells,GPC	*S.aureus*	R			S	S	R			S	S	S	S	S	R				NT	S					RD
89	26	M	85112	OP	–	–	–	–	–	yes	yes	CAP	LUL	SP	Few pus cells &no organism	CNP																								LF
														B		NG																								
90	52	F	85231	OP	DM/ HT	–	–	–	–	no	no	CAP	LUL	SP	Few pus cells &no organism	CNP																								LF
														B		NG																								
91	69	M	85345	OP	DM	–	–	–	–	yes	yes	CAP	L LL	SP	BS +1,pus cells,GNB	S.marcesens	R		S			R	S	S	S	S	S	S	R	S	S	S	S		NT					RD
92	49	M	85467	OP	DM	–	–	–	–	yes	yes	CAP	R ML	SP	Few pus cells &no organism	CNP																								LF
														B		NG																								
93	42	F	10599	MW	APD /UGI Bleeding	yes	yes	–	–	no	no	HAP	B/L	SP	BS +1,pus cells,GPC	S.aureus	R		R	S	R					S	S		S	R				NT	S					RD
94	50	M	85543	OP	DM /HT	–	–	–	–	yes	yes	CAP	R ML	SP	Few pus cells &no organism	CNP																								LF
														B		NG																								
95	23	M	85642	OP	–	–	–	–	–	yes	yes	CAP	L LL	SP	Few pus cells &no organism	CNP																								LF
														B		NG																								
96	64	M	85676	OP	DM	–	–	–	–	yes	yes	CAP	L LL	SP	BS +1,pus cells,GPC	*S.aureus*	R			S	S	R			S	S	S	S	S	R				NT	S					LF
97	48	M	85699	OP	DM	–	–	–	–	yes	yes	CAP	L LL	SP	Few pus cells &no organism	CNP																								LF
														B		NG																								
98	47	F	85714	OP	DM/ HT	–	–	–	–	no	no	CAP	R UL	SP	Few pus cells &no organism	CNP																								LF
														B		NG																								
99	62	M	85765	OP	DM /HT	–	–	–	–	yes	yes	CAP	R UL	SP	BS +1,pus cells,GNB,	*K.pneumoniae*	R		S			S	S	S	S	S	R	S	S	R	S	S	S		S					LF
														B		*K.pneumoniae*	R		S			S	S	S	S	S	R	S	S	R	S	S	S		S					
100	63	M	85799	OP	DM	–	–	–	–	yes	yes	CAP	R UL	SP	BS +1,pus cells,GNB,GPC	*S.aureus*	R			S	S	R			S	S	S	S	S	R				NT	S					LF
																K.pneumoniae	R		S			R	S	S	S	R	S	R	R	R	S	S	S		S					
101	66	M	85854	OP	DM	–	–	–	–	yes	yes	CAP	R LL	SP	Few pus cells &no organism	CNP																								LF
														B		NG																								
102	21	M	85876	OP	–	–	–	–	–	yes	yes	CAP	R LL	SP	Few pus cells &no organism	CNP																								LF
														B		NG																								
103	65	F	85897	OP	–	–	–	–	–	no	no	CAP	R LL	SP	Few pus cells &no organism	CNP																								LF
														B		NG																								
104	64	M	85923	OP	DM	–	–	–	–	yes	yes	CAP	R UL	SP	BS +1,pus cells,GNB	*K.pneumoniae*	S		S			S	S	S	S	R	R	R	S	R	S	S	S		S					RD
																P.aeroginosa	R		S			NT	NT	S	S	S	R	S	R		S	S	S		NT					

105	65	M	85946	OP	DM /HT	–	–	–	–	yes	yes	CAP	R UL	SP	BS +1,pus cells,GPC,GNB	*E.coli*	R		S		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S		S					RD
																K.pneumoniae	R		S			S	S	S	S	S	S	S	S	S	S	S	S	S	S		S					
106	22	M	85966	OP	–	–	–	–	–	yes	yes	CAP	L LL	SP	Few pus cells &no organism	CNP																										LF
														B		NG																										
107	64	M	85987	OP	DM	–	–	–	–	no	no	CAP	L LL	SP	BS +1,pus cells,GNB	*K.pneumoniae*	R		R			S	S	S	S	S	S	S	S	R	S	S	S	S	S		S					RD
108	60	M	115264	IMCU	HT/CVA	YES	YES	yes	–	yes	yes	VAP	B/L	E	pus cells,GNB	E.coli	R		R				S	S	R	R	R	R	R		R	R	S	S	S		R			**AMPC**		EX
														B		NG																										
109	43	M	86012	OP	DM	–	–	–	–	yes	no	CAP	R ML	SP	BS +1,pus cells,GNB	*K.pneumoniae*	S		R			S	S	S	S	S	S	S	R	R	R	R	S	S	S		S					RD
														B																												
110	69	M	86045	OP	DM /COPD	–	–	–	–	yes	no	CAP	R LL	SP	BS +1,pus cells,GNB	*K.pneumoniae*	R		S			S	S	S	S	S	S	S	S	S	R	R	S	S	S		S					RD
111	23	F	86057	OP	–	–	–	–	–	no	no	CAP	B/L	SP	Few pus cells &no organism	CNP																										LF
														B		NG																										
112	42	M	86089	OP	DM	–	–	–	–	yes	no	CAP	R ML	SP	BS +1,pus cells,GNB	*E.coli*	S		S		R	S	S	S	S	S	S	S	S	S	S	R	S	S	S		S					RD
														B		NG																										
113	69	M	86123	OP	DM	–	–	–	–	no	yes	CAP	L LL	SP	BS +1,pus cells,GPC	*S.aureus*	R			R	S	R			S	S	S	S	S	S	S	R				NT	S					RD
														B		NG																										
114	41	F	86145	OP	– /COPD	–	–	–	–	no	no	CAP	R LL	SP	BS +1,pus cells,GNB	*K.pneumoniae*	R		S			S	S	S	S	S	S	S	S	S	S	R	S	S	S		S					RD
														B		NG																										
115	40	M	86198	OP	COPD	–	–	–	–	no	yes	CAP	R LL	SP	BS +1,pus cells,GNB	*K.pneumoniae*	R		R			S	S	S	S	R	S	R	S	S	S	R	S	S	S		S					RD
														B		NG																										
116	69	M	86234	OP	DM	–	–	–	–	no	yes	CAP	R UL	SP	BS +1,pus cells,GPC	CONS	R			S	S	R	S			S		S	R	S	R	R				NT	S					EX
117	61	M	10699	ORTHO	DM/BOTH #FOREARM	yes	yes	–	–	no	no	HAP	B/L	SP	BS +1,pus cells,GPC	CONS	R		S	S	S					S		S	R		S	R				NT	S					RD
118	39	F	86324	OP	–	–	–	–	–	no	no	CAP	R ML	SP	Few pus cells &no organism	CNP																										LF
														B		NG																										
119	68	M	86367	OP	DM	–	–	–	–	no	no	CAP	R ML	SP	BS +1,pus cells,GNB	P.aeroginosa	NT		R			NT	NT	S	S	S	S	S	S				S	S	S		NT					EX
120	67	M	86432	OP	DM	–	–	–	–	yes	no	CAP	L LL	SP	BS +1,pus cells,GPC	*S.aureus*	R			S	S	R			S	S	S	S	S	S	S	R				NT	S					EX
121	23	M	10789	ORTHO	#hand	yes	yes	–	–	no	no	HAP	L LL	SP	BS +1,pus cells,GPC	S.aureus	R		R	R	S					S		S	R		R	R				NT	S					RD
122	62	F	86487	OP	DM /COPD	–	–	–	–	no	no	CAP	R UL	SP	BS +1,pus cells,GNB	*K.pneumoniae*	R		S			R	S	S	S	R	S	R	R	S	S	S	S	S	S		S					EX
																CONS	R			S	S	R	S			S		S	R	S	R	R				NT	S					
123	67	M	86534	OP	DM	–	–	–	–	yes	yes	CAP	R ML	SP	BS +1,pus cells,GPC IN PAIRS	S.pneumoniae	R	S		S	S	R	S			S		S	S	S	R	R				NT						LF
124	24	M	86576	OP	–	–	–	–	–	yes	yes	CAP	LUL	SP	Few pus cells &no organism	CNP																										LF
														B		NG																										
125	61	F	86588	OP	DM/HT	–	–	–	–	no	no	CAP	R UL	SP	BS +1,pus cells,GPC,GNB	*S.aureus*	R			S	S	R	S		S	S	S	S	R	S	S	R				NT	S					LF
																K.pneumoniae	R																									
126	66	M	86599	OP	DM	–	–	–	–	yes	yes	CAP	RUL	SP	BS +1,pus cells,GPC,GNB	*K.pneumoniae*	R		S			R	S	S	S	R	S	R	S	S	S	R	S	S	S		S					LF
																CONS	R			S	S	R	S		S	S	S	S	R	S	S	R				NT	S					

127	62	M	86604	OP	DM/HT	–	–	–	–	yes	yes	CAP	R UL	SP	BS +1,pus cells,GNB	*E.coli*	R		S		S	S	S	S	S	S	S	S	S	S	S	S	S		S					LF
128	55	F	86615	OP	–	–	–	–	–	no	no	CAP	R ML	SP	Few pus cells &no organism	CNP																								LF
														B		NG																								
129	34	M	86624	OP	COPD	–	–	–	–	yes	yes	CAP	B/L	SP	BS +1,pus cells,GNB	*K.pneumoniae*	R		R			R	S	S	S	R	R	S	S	R	S	S	S		S					RD
130	63	M	86645	OP	DM	–	–	–	–	yes	no	CAP	L LL	SP	BS +1,pus cells,GNB	*K.pneumoniae*	R		R			R	S	S	S	S	R	S	R	R	S	S	S		S					LF
131	33	F	86678	OP	–	–	–	–	–	no	no	CAP	R UL	SP	Few pus cells &no organism	CNP																								LF
														B		NG																								
132	25	M	10876	IMCU	COPD	yes	yes	–	–	no	no	HAP	R DIFFUSE	SP	Few pus cells &no organism	CNP																								LF
														B		NG																								
133	44	F	86745	OP	DM	–	–	–	–	no	no	CAP	R UL	SP	BS +1,pus cells,GPC	*S.aureus*	R			S	S	R			S	S	R	S	R	R				NT	S					RD
134	32	F	86834	OP	–	–	–	–	–	no	no	CAP	LUL	SP	Few pus cells &no organism	CNP																								LF
														B		NG																								
135	41	M	86897	OP	–	–	–	–	–	yes	no	CAP	LUL	SP	Few pus cells &no organism	CNP																								LF
														B		NG																								
136	52	F	86956	OP	–	–	–	–	–	no	no	CAP	R UL	SP	Few pus cells &no organism	CNP																								LF
														B		NG																								
137	67	M	86976	OP	DM	–	–	–	–	yes	no	CAP	LUL	SP	BS +1,pus cells,GNB	*K.pneumoniae*	R		S			R	S	S	S	S	S	S	R	R	S	S	S		S					LF
138	33	M	86999	OP	HT/COPD	–	–	–	–	yes	no	CAP	R ML	SP	BS +1,pus cells,GNB	*K.pneumoniae*	R		S			R	S	S	S	S	S	S	S	R	S	S	S		S					RD
139	25	M	87000	OP	–	–	–	–	–	yes	no	CAP	B/L	SP	Few pus cells &no organism	CNP																								LF
														B		NG																								
140	39	F	10976	SW	appendicitis/copd	yes	yes	–	–	no	no	HAP	LUL	SP	Few pus cells &no organism	CNP																								LF
														B		NG																								
141	45	M	87015	OP	DM/HT	–	–	–	–	yes	no	CAP	R LL	SP	BS +1,pus cells,GNB	P.aeroginosa	NT		R			NT	NT	S	S	R	R				S	S	S		NT					RD
142	34	M	87045	OP	–	–	–	–	–	no	no	CAP	R UL	SP	Few pus cells &no organism	CNP																								LF
														B		NG																								
143	26	M	87068	OP	–	–	–	–	–	no	no	CAP	L LL	SP	Few pus cells &no organism	CNP																								LF
														B		NG																								
144	65	F	87098	OP	DM	–	–	–	–	no	no	CAP	L LL	SP	BS +1,pus cells,GNB	*E.coli*	R		S		R	S	S	S	S	S	S	S	S	R	S	S	S		S					LF
145	35	M	87134	OP	–	–	–	–	–	no	yes	CAP	R ML	SP	Few pus cells &no organism	CNP																								LF
														B		NG																								
146	66	M	87145	OP	DM/COPD	–	–	–	–	no	no	CAP	R UL	SP	Few pus cells &no organism	CNP																								LF
														B		NG																								
147	45	M	16653	IMCU	HE/CLD	YES	YES	yes	–	yes	yes	VAP	R DIFFUSE	E	pus cells,GPC clusters	S.aureus	R		R	R	R					S	R		R	R				NT	R	**MRSA**				EX
														B		NG																								
148	48	M	16202	IMCU	HT/COPD	YES	YES	–	yes	yes	no	VAP	R LL	E	Pus cells,GNB	p,aeruginosa	R		R				S	S	S	S	S		S		S	S	S		S					RD
														B		NG																								

149	65	M	87245	OP	DM /HT	–	–	–	–	no	no	CAP	R ML	SP	BS +1,pus cells,GPC,GNB	*K.pneumoniae*	R		S			R	S	S	S	S	S	S	S	R	S	S	S		S					RD
																S.aureus	R		R	R	R	R				S	R		R	R				N T	R	M R S A				
150	28	F	18210	IM CU	R K POI SON ING	YES	-	–	y e s	no	no	VAP	R LL	E	pus cells,GNCB	A,baumanii	R		S				S	S	S	S	R		R		S	S	S		S					RD
														B		NG																								
151	71	M	87321	OP	DM	–	–	–	–	no	no	CAP	B/L	SP	BS +1,pus cells,GNB	*K.pneumoniae*	R		S			R	S	S	S	S	S	R	S	S	S	S	S		S					EX
152	57	M	87356	OP	DM /CO PD	–	–	–	–	no	no	CAP	L LL	SP	BS +1,pus cells,GNB	*K.pneumoniae*	R		S			R	S	S	S	S	S	R	S	R	S	S	S		S					RD
153	38	M	17321	IM CU	OPC POI SON ING	YES	YES	–	y e s	yes	yes	VAP	R ML	E	pus cells,GNCB	K.pneumoniae	R		S				R	R	R	R	R		R	R	S	S	S		S		**ESB L**			EX
														B		K.pneumoniae	R		S				S	S	S	S	S	S	S	S	S	S	S		S					
154	37	M	87435	OP	–	–	–	–	–	no	yes	CAP	R ML	SP	Few pus cells &no organism	CNP																								LF
														B		NG																								
155	38	M	87897	OP	–	–	–	–	–	yes	no	CAP	R ML	SP	Few pus cells &no organism	CNP																								LF
														B		NG																								
156	71	M	17861	IM CU	HT/ DM/ ME	YES	-	yes	–	yes	yes	VAP	B/L	E	pus cells,GNCB	A.baumanii	R		R				S	S	S	S	R		R		S	S	S		S					RD
														B		NG																								
157	31	M	11123	M W	ascit es/H T/D M	yes	yes	–	–	yes	yes	HAP	R ML	SP	BS +1,pus cells,GNB	K.pneumoniae	R		R				R	R	R	S	R		R	R	S	S	S		S		ESB L			EX
158	69	M	88012	OP	DM /CO PD	–	–	–	–	yes	no	CAP	B/L	SP	BS +1,pus cells,GPC	*S.aureus*	R		R	R	R	R			R	R	R	R	R	R				N T	R	M R S A				RD
159	43	F	11145	OR TH O	# r clavi cle /DM	yes	yes	–	–	no	no	HAP	B/L	SP	Few pus cells &no organism	CNP																								LF
														B		NG																								
160	62	M	11265	T M W	DM /bro nchit is	yes	yes	–	–	yes	no	HAP	L ML	SP	BS +1,pus cells,GNB	K.pneumoniae	R		R				S	S	S	S	S		S	S	S	S	S		S					RD
161	31	F	88123	OP	–	–	–	–	–	no	no	CAP	R ML	SP	Few pus cells &no organism	CNP																								LF
														B		NG																								
162	50	F	17962	IM CU	HT/ PE	YES	YES	–	y e s	no	no	VAP	R LL	E	Pus cells,GNB	p,aeruginosa	R		R				S	S	S	S	R		R		S	S	S							RD
														B		NG																								
163	65	M	11287	M W	LRT I	yes	yes	–	–	yes	no	HAP	B/L	SP	BS +1,pus cells,GNB,GPC	E.coli	R		S				S	S	S	S	R		R	R	S	S	S		S					RD
																E.fecalis	R		S	R	S					S	S(HL G120)		R	R				S						
164	27	F	12134	T M W	BA	yes	yes	–	–	no	no	HAP	B/L	SP	BS +1,pus cells,GNB	K.pneumonia	R		R				R	R	R	R	R		R	R	S	S	S		R			AM P C		RD
																A.baumanii	R		R				S	S	S	S	R		R		S	S	S		S					
165	63	M	17344	IM CU	DM/ HT/ CK D	YES	YES	yes	–	yes	no	VAP	B/L	E	pus cells,GNB	K.pneumoniae	R		S				S	S	S	S	S		S	R	S	S	S		S					EX
														B		NG																								
166	56	M	12354	OR TH O	DM/ HT/ R AN KLE DIS	yes	yes	–	–	yes	yes	HAP	B/L	SP	BS+1,Pus cells,GNB,	K.pneumonia	R		R				R	R	R	S	R		R	R	S	S	S		S		ESBL			EX
167	40	M	88567	OP	COP D	–	–	–	–	no	yes	CAP	R ML	SP	BS+1,Pus cells,GNB,	*K.pneumoniae*	R		R			R	S	S	S	S	R	R	S	R	S	S	S		S					RD
168	42	F	12654	T M W	COP D /	yes	yes	–	–	no	no	HAP	R LL	SP	BS+1,Pus cells,GNB,	P.aeroginosa	R		S				S	S	S	S	R		R		S	S	S							RD
169	62	M	88765	OP	DM /HT	–	–	–	–	no	no	CAP	B/L	SP	BS+1,Pus cells,GNB,	*K.pneumoniae*	R		R			R	S	S	S	S	R	R	R	R	S	S	S		S					RD
170	61	M	88890	OP	DM	–	–	–	–	no	no	CAP	R ML	SP	BS+1,Pus cells,GNB,	*E.coli*	R		S		S	S	S	S	S	S	S	S	S	S	S	S	S		S					RD

171	36	F	19362	IM CU	OPC POI SON ING	YES	-	yes	–	no	no	VAP	B/L	E	pus cells,GPC clusters	S.aureus	R		R	S	S					R	R		R	R				N T	S					RD
														B		NG																											
172	68	M	12765	IM CU	DM /CV A	yes	yes	–	–	yes	yes	HAP	B/L	SP	BS+1,Pus cells,GNB,	P.aeroginosa	R		S				S	S	S	R	S	S	S		S	S	S		S				RD
173	58	M	12876	OR TH O	DM / THR	yes	yes	–	–	yes	yes	HAP	R DIF FUS E	SP	BS+1,Pus cells,GNB,	E.coli	R		R				R	S	S	S	S	S	R	S	S	S	S		S				RD
174	41	F	89000	OP	–	–	–	–	–	no	no	CAP	B/L	SP	Few pus cells &no organism	CNP																								LF
														B		NG																											
175	63	M	12965	M W	DM /HT /ALD	yes	yes	–	–	yes	no	HAP	B/L	SP	BS +1,Pus cells ,GNB ,GPC	CONS	R		S	R	S					S	R		R	S				N T	S					RD
																K.pneumonia	R		R				S	S	S	R	R		R	R	S	S	S		S				RD
176	36	M	12999	OR TH O	R Shoulder dislocation/DM	yes	yes	–	–	yes	yes	HAP	B/L	SP	BS+1,Pus cells,GNB,	K.pneumonia	R		R				S	S	S	S	S		S	R	S	S	S		S				RD
177	60	M	12386	IM CU	DM/ CA D/C VA	YES	YES	yes	–	yes	yes	VAP	R LL	E	pus cells,GNB	P.aeroginosa	R		R				R	S	R	R	R		R		S	R	R		S			**MBL**	EX
														B		NG																											
178	42	M	89123	OP	DM /COPD	–	–	–	–	no	no	CAP	R ML	SP	BS+1,Pus cells,GNB,	*A.baumanii*	R		S			R	S	S	S	S	R	S	R		S	S	S		N T				RD
179	60	M	12585	IM CU	HT/ PE	YES	YES	yes	–	yes	yes	VAP	B/L	E	pus cells,GNB	E.coli	R		S				S	S	S	S	S		S	R	S	S	S		S				EX
														B		NG																											
180	47	M	89342	OP	DM /COPD	–	–	–	–	no	no	CAP	R ML	SP	BS+1,Pus cells,GPC	*S.aureus*	R			R	R	R			R	R	R	R	R	R				N T	R	M RSA				RD
181	55	F	123681	IM CU	HT/ COPD	YES	-	yes	–	no	no	VAP	R ML	E	pus cells,GNB	K.pneumoniae	R		R				R	R	R	S	R	R	R	R	S	S	S		R			**AMPC**		RD
														B		K.pneumoniae	R		S			R	S	S	S	S	S	S	S	S	S	S	S		S					
182	47	F	126761	IM CU	HT/ CV A	YES	YES	–	yes	no	no	VAP	B/L	E	pus cells,GNCB	A,baumanii	R		S				S	S	S	R	R			R	S	S	S		S				RD
														B		NG																											
183	61	M	13121	IM CU	/ TIA	yes	yes	–	–	yes	yes	HAP	B/L	SP	BS+1,Pus cells,GNB,	E.coli	R		S				S	S	S	S	R		R	S	S	S	S		S				RD
184	52	M	13165	T M W	DM / BRONCHITIS	yes	yes	–	–	no	no	HAP	L ML	SP	BS+1,Pus cells,GNB,	K.pneumoniae	R		R				S	S	S	S	S		R	R	S	S	S		S				RD
														B		K.pneumoniae	R		S				S	S	S	S	S		R	S	S	S	S		S					
185	52	M	125861	IM CU	DM/ HT/ CKD	YES	YES	–	yes	yes	no	VAP	B/L	E	Pus cells,GNB	E.coli	R		S				S	S	S	R	R		R	R	S	S	S		S				RD
														B		NG																											
186	65	F	89534	OP	DM/ HT	–	–	–	–	no	no	CAP	R UL	SP	BS+1,Pus cells,GNB,GPC	*CONS*	R			S	S	R			R	S	R	S	R	R				N T	S					EX
																K.pneumoniae	R		S																									
187	70	M	89643	OP	DM /COPD	–	–	–	–	no	no	CAP	B/L	SP	BS+1,Pus cells,GNB,	*K.pneumoniae*	R		S			R	S	S	S	S	R	R	R	R	S	S	S		S				EX
188	50	M	12896	IM CU	HT/ DCL D/D M	YES	YES	yes	–	yes	yes	VAP	R LL	E	pus cells,GNB	P.aeroginosa	R		R				R	S	R	R	R		R		S	R	R		S			**MBL**	EX
														B		NG																											
189	43	M	23967	IM CU	DM/ HT/ ME	YES	YES	yes	–	yes	no	VAP	R DIF FUS E	E	pus cells,GNB	E.coli	R		S				S	S	S	S	R		R	R	S	S	S		S				EX
														B		NG																											
190	48	M	24400	IM CU	OPC POI SON ING	YES	YES	yes	–	yes	yes	VAP	L LL	E	pus cells,GNCB	A.baumanii	R		S				S	S	S	R	S		S		S	S	S		S				RD
														B		NG																											

191	69	M	13199	ORTHO	DM / # femur L	yes	yes	–	–	yes	yes	HAP	R ML	SP	BS+1,Pus cells,GNB,	Proteus mirabilis	R		S				S	S	S	S	R		R	R	S	S	S		S					RD
192	69	M	24764	IMCU	DM/HT/CKD/PE	YES	YES	yes	–	yes	yes	VAP	B/L	E	pus cells,GNB,GPC	K.pneumoniae	R		S				S	S	S	S	S		R	R	S	S	S		S					EX
																S.aureus	R		R	S	S					S	S		S	R				NT	S					
														B		NG																								
193	38	F	13265	OG	DUB /DM	yes	yes	–	–	no	no	HAP	B/L	SP	BS+1,Pus cells,GNB,	K.pneumoniae	R		R				R	R	R	R	R		R	R	S	S	S		S		ESBL			RD
																A.baumanii	R		R				S	S	S	R	R		R		S	S	S		S					
194	25	F	266441	IMCU	R K POISONING	YES	YES	–	yes	no	no	VAP	R LL	E	Pus cells,GNB	p,aeruginosa	R		R				S	S	S	S	R		R		S	S	S		S					RD
														B		NG																								
195	61	F	89941	OP	DM	–	–	–	–	no	no	CAP	R ML	SP	BS+1,Pus cells,GNB,	*K.pneumoniae*	R		S			R	S	S	S	S	S	R	R	R	S	S	S		S					EX
196	43	F	89954	OP	DM /COPD	–	–	–	–	no	no	CAP	B/L	SP	Few pus cells &no organism	CNP																								LF
														B		NG																								
197	64	M	89965	OP	DM	–	–	–	–	no	yes	CAP	R LL	SP	BS+1,Pus cells,GNB	*K.pneumoniae*	R		S			R	S	S	S	R	S	S	S	S	S	S	S		S					RD
																A.baumanii	R		S			R	S	S	S	S	S	S	S		S	S	S							
														B		NG																								
198	68	M	89978	OP	DM	–	–	–	–	no	no	CAP	R LL	SP	BS+1,Pus cells,GNB,GPC	*K.pneumoniae*	R		R			R	S	S	S	R	S	S	S	R	S	S	S		S					RD
																S.aureus	S		S	S	S					S	S		S	S				NT	S					RD
199	65	F	89986	OP	DM /COPD	–	–	–	–	no	no	CAP	R ML	SP	BS+1,Pus cells,GNB,	P.aeroginosa	NT		S			NT	NT	S	S	S	R				S	S	S		NT					EX
200	45	M	27643	IMCU	HT/CVA	YES	YES	yes	–	yes	no	VAP	R ML	E	pus cells,GNB	K.pneumoniae	R		S				R	R	R	R	R		R	R	S	S	S		S		**ESBL**			EX
														B		NG																								
201	29	F	13654	SW	LIPOMA	yes	yes	–	–	no	no	HAP	B/L	SP	BS+1,Pus cells,GNB,	E.coli	R		R				R	R	R	R	R		R	R	S	S	S		R			**AMP C**		EX
202	40	F	29693	IMCU	HT/COPD	YES	YES	–	yes	no	no	VAP	B/L	E	pus cells,GPC clusters	S.aureus	R		R	R	R					R	R		S	R				NT	S					RD
														B		NG																								
203	35	F	900223	OP	/COPD	–	–	–	–	no	no	CAP	B/L	SP	BS+1,Pus cells,GNB,	*K.pneumoniae,*	R		S			R	S	S	S	R	S	S	S	R	S	S	S		S					RD
																E.coli																								
204	41	F	900342	OP	/COPD	–	–	–	–	no	no	CAP	R LL	SP	BS+1,Pus cells,GNB,	*K.pneumoniae*	R		R			R	S	S	S	R	S	R	S	R	S	S	S		S					RD
205	37	F	900412	OP	/COPD	–	–	–	–	no	no	CAP	B/L	SP	BS+1,Pus cells,GNB,	*K.pneumoniae*	R		S			R	S	S	S	R	S	R	S	R	S	S	S		S					RD
														B			R		S																					